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Preface

The launching of a second edition of a warmly received textbook on Practical Physiology is in some ways more of a challenge than the preparation of the first edition. It is not merely enough to match the standard of the first, rather it ought to be surpassed. To what extent I have succeeded in achieving this goal, only the readers and time will tell.

The material included within the covers of this book conforms to the syllabi and courses of various medical colleges in this country. The book is divided onto 3 sections : amphibian, mammalian, and human. Though there is continuing controversy and divergence of opinion regarding the necessity of including amphibian experiments in the medical curriculum, they have to be included in a book meant primarily for Indian medical students till such times when the courses are modified. Although they appear to be time wasting and irrelevant to clinical medicine they do serve a useful purpose. These experiments train the students to work with their hands, make careful observations, critically analyse the results and draw logical conclusions. These are qualities that the students will depend upon later in their clinical work. A compromise can, however, be made ; the number of experiments to be done by the students themselves may be reduced while the rest are demonstrated to them in small batches. There is no second opinion regarding the human experiments.

The chief aim of the book remains the same as before, that is to say, to help the fresh medical students in coping with the day-to-day problems arising from handling the various apparatuses and carrying out experimental work. If the student has a hazy notion of the purpose of the experiment and the correct way of carrying it out, he will be easily disheartened and frustrated. I hope to help the student with a clear idea of what he is expected to do and a more definite plan of doing it.

In general the approach of the text has not been altered. However, considerable revision has been undertaken. There are 26 new figures and diagrams and most of the original ones

have been replaced for better clarity. In essence, each experiment begins with the principle on which it is based and the apparatus required for it. Then follows the step-by-step 'procedure' in which the working instructions are so framed that the student should find no difficulty in tackling any experiment. Next come the 'observations' results and conclusions'. The relevant theoretical aspects that are needed for immediate reference, including deviations from normal, are then described under the heading of 'discussion'. This is intended to obviate the necessity for the student to refer to textbooks on physiology again and again. Finally, the questions generally asked from the students are grouped at the end of each experiment. A student should be able to assess his comprehension of the relevant subject matter in attempting to answer these questions. The appendix contains a comprehensive list of normal standards, and normal physiological and biochemical values. These will certainly prove useful to the harried and hurried medical student.

It is a pleasure to acknowledge the encouragement extended to me by my many friends, particularly-Dr.O.P.Mahajan, Dr. Mrs B.K.Pannu, Dr. Mrs G.K. Ahuja, Dr.S.Mookerjee, Dr.A.P. Sharma, Dr.G.L. Dhar, Dr. G.M. Shah Dr. M.Sayeed, Dr.O.P. Tandon, Dr. Mrs. B.K. Maini, Dr. N.K. Mishra, Dr. U.C. Rai, Dr. S.K. Manchanda, Dr. R.K. Marya, and Dr. S.V.Rao. They have been generous enough with their suggestions whenever I met them or wrote to them. I am also grateful to my colleagues Dr. Mrs. P. Khetarpal, Dr. Kanta Kumari, Dr. R S. Sidhu, Dr. Ram Sarup, and Dr. Parveen Gupta for their help and cooperation.

I am much indebted to Mr. Jitendar P Vij, my publisher, for his continued cooperation, enthusiasm, and excellent work in bringing out this edition.

May this book act as an effective stimulus for the students to gain first-hand knowledge in Physiology and ease his journey through a complex but fascinating science. If the work seems difficult at times he must not give up. As he gathers experience, the path will become easier. The discipline of work will then become the most exciting and rewarding experience in life.

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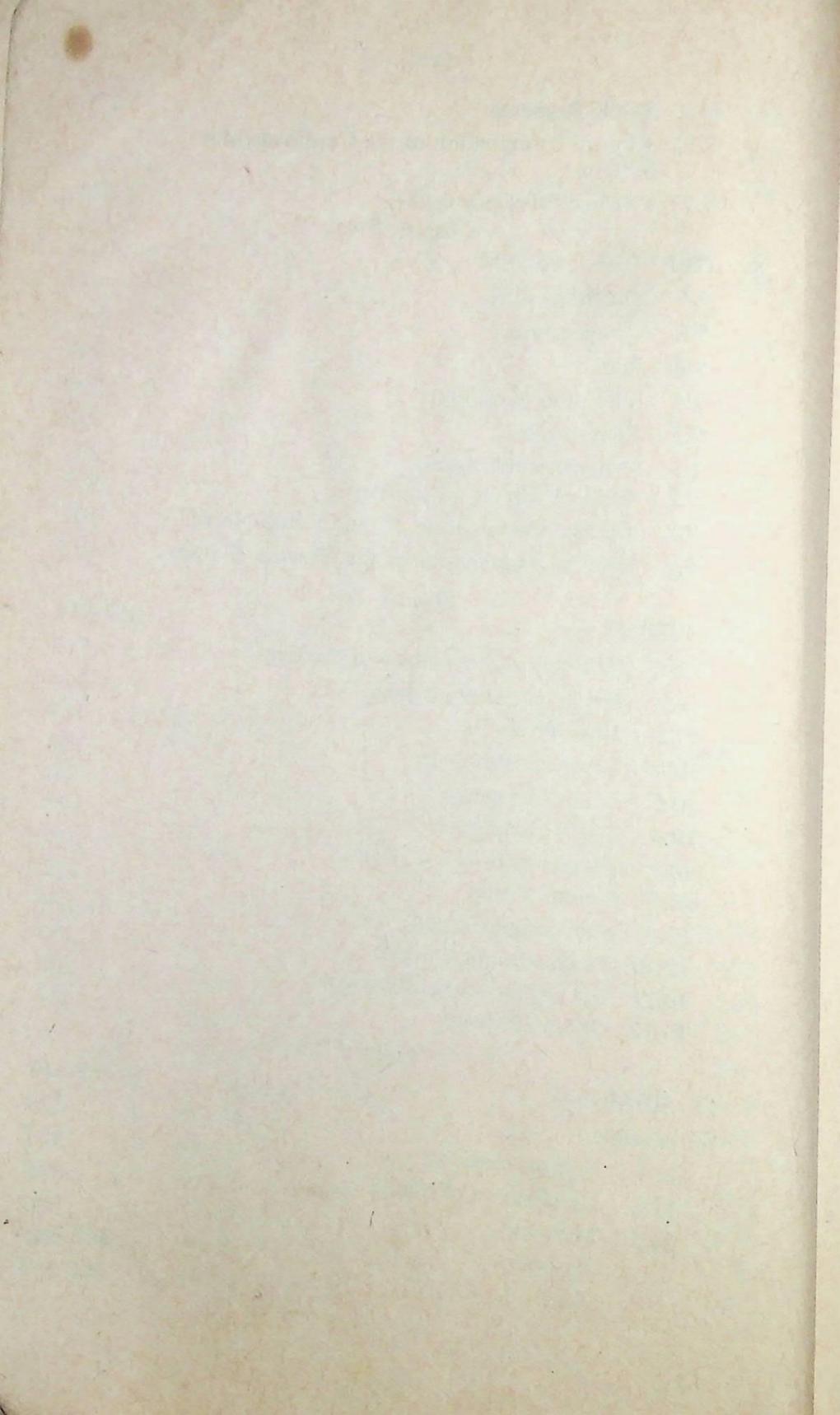
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General Introduction

The term "physiology" is derived from a Greek root with the Latin equivalent *physiologia*, originally meaning "Natural knowledge". Though used for the first time by Jean Fernel in 1542, the word did not come into common use until the 19th century. The subject of physiology now refers to the study of phenomena exhibited by living organisms, the classification of these phenomena, their sequential occurrence and relative importance, the allocation of each function to its appropriate organ, and the communication and control systems which coordinate the various systems. The basic theme of physiology is the interaction between a living organism and its environment, that is to say, how the organism reacts as an integrated unit to changes in its environment.

If we trace the evolution of physiology, we find that it is intimately linked to the growth of medicine—the mother of all branches of natural sciences. Chemistry, physics, botany, zoology, pathology, microbiology and all their branches, have all evolved from the study of the art of healing. The relationship between physiology on the one hand, and chemistry, physics, and morphological sciences like anatomy and histology on the other, so intimate at present, extends back through the centuries to the time of Galen (AD—131—201). Galen's theory of "spirits" and other teachings went unchallenged for over 14 centuries until the time of Vesalius (1514—1564), the father of Anatomy, and Harvey (1578—1667), the father of Physiology. At about this time (17th century), the science of physics, and later, that of chemistry, began to take shape and though the next two centuries were rich in the accumulation of isolated facts, the physiological knowledge advanced rather slowly. Thus, though the microscope had been invented by Galileo in 1609 it was not

until over half a century later that it was used extensively by Antony Leeuwenhoek (1715), a Dutchman and a town clerk by profession, and others. Some of the discoveries made during 17th, 18th and 19th centuries, however, laid the foundations of experimental physiology on sound footings. The discovery that the blood travels around in a circuit in the body was made by William Harvey in 1628 ; Malpighii discovered capillaries in the frog's lung in 1661 (thus completing the circuit of blood discovered by Harvey) ; Stephen Hales first measured the blood pressure in a mare in 1733 ; Bernoulli's work led to the study of movement of fluids ; Priestley and Scheele independently discovered oxygen ; Lavoisier unveiled the true nature of combustion between 1775 and 1794 ; and Henry Cavendish discovered the composition of water in 1781, and that of air in 1784. The invention of Leyden jar about the middle of 18th century provided an admirable stimulus for excitable tissues and Galvani (1786) made original observations on animal electricity and electrical nature of conducted processes in nerves. The invention of mercury manometer by Poiseuille in 1825, of galvanometer before mid 19th century, and of induction coil by Du Bois Reymond in 1849 are other landmarks in the field of experimental physiology.

A characteristic feature of research in experimental physiology done upto about mid 19th century was that most scientists worked singly and without any collaboration. There were no special physiological laboratories and almost no physiological apparatuses, the only laboratory equipment available being a microscope, ordinary scales, thermometers, some simple electrical equipment and laboratory glassware. Even then the work done by various researchers is deserving of considerable merit and should be a source of inspiration to medical students. The wide array of mechanical, optical, and electronic instruments now available in a laboratory reflects the ingenuity of man in bringing our knowledge of physiology to its present state.

It is interesting to note that many of the outstanding physiologists have been practicing physicians. We are now aware of the tremendous body of physiological knowledge that has its origin in the study of disease. In turn, the exciting progress in

medicine in this century has largely originated from the application of physiology to clinical medicine. Although animal experimentation has tremendously enriched physiology, nature sometimes poses problems which are difficult, if not impossible, to duplicate in experimental animals, and which, therefore, must be studied in man. Diseases, in this sense, are experiments of nature.

Organism and Environment :

The interaction between a living organism and its environment is a fundamental concept of physiology. The activities of an organism must be studied in relation to its environment the chief components of which are : water, gases, temperature, gravity, food materials including organic and inorganic substances, and of course, other organisms. Living organisms have many attributes amongst which excitability (i.e., the ability to respond appropriately to a change in the environment, that is to say to a stimulus), organisation, food intake and expenditure of energy, growth and repair, movement and reproduction are the most evident. Living cells are dynamic entities and they persist not in spite of but because of the constant chemical changes occurring within them. This dynamic stability of the living cells, a result of a delicate balance between the processes of repair (anabolism) and breakdown (catabolism), allows the organism to interact with its environment.

In order that an organism may survive, there are three courses open to it : it must adapt itself to the environment, or it must move away from it if it is so hostile that adaptation is not possible, or it must change the environment. If neither of these responses is possible, the organism must perish and cease to exist as an independent unit. All these responses have been utilized by animals and man in the course of evolution.

In simpler organisms the range and power of adaptation responses are limited. However, in complex organisms, due to the selective processes of evolution, more flexible memory stores have evolved, the result being that a choice of a particular response can be made from amongst many alternatives available. Amoeba, a unicellular organism, is very limited in its responses

because the same cell has to perform the functions of ingestion and digestion, excretion, movement and reproduction. In complex organisms, on the other hand, various organs and organ systems have come to be set apart for the performance of such diverse functions as ingestion, digestion, excretion, movement, circulation, respiration and so on. This specialisation has, therefore, considerably increased the chances of survival of such organisms.

The Internal Environment of the Body

Life is believed to have originated in the seas, which therefore, formed the external environment of early forms of life. While unicellular and few-celled organisms could exchange oxygen and other nutrients as well as waste products directly with the external or general environment, this process could not operate in multicellular organisms in which most of the cells were located deep within the body. But if these cells could not gain access to the sea, the sea would have to be brought to them. Each cell in the depths of the body would then be bathed by a fluid with which it could enter into exchanges. This is exactly what is believed to have happened. As evolution proceeded, the external environment was 'internalized' and the sea became the tissue fluid or the extracellular fluid (ECF). The evolution of ECF is evident from its composition—it has more sodium, chloride, and bicarbonate as compared to the intracellular fluid which has more potassium, magnesium, phosphate and proteins. The cell membranes, because of their selective permeabilities, keep the two fluid compartments separated from each other.

The very thin film of tissue fluid, though lying outside each cell and having no direct contact with the external environment, is called "the internal environment of the body" or simply the "internal environment". Claude Bernard (1857), a French physician and a great experimental physiologist, employed the term "millieu intérieur" for the internal environment. He pointed out that for the normal functioning of the cells, and therefore of the whole body, the composition of tissue fluid must be maintained within very narrow limits. The common aim of all the organs and organ systems in a complex organism, including

man, is to contribute in some way to the stability of the internal environment. Walter Cannon (1897) used the term "homeostasis" to mean the condition of uniformity or constancy of the internal environment that results from the adjustments of living things to changes in their environment. All adaptation responses exhibited by living organisms are therefore homeostatic responses.

Body as a Cell State

The body of a complex organism contains millions and millions of cells performing different functions. In mid 19th century, Rudolf Virchow, a French pathologist, pointed out that the organisation of cells in a complex organism resembles in many ways the organisation of people within the human society. Besides performing some functions that are common to all, most people work at a particular job or profession almost exclusively. The great advantage of this division of labour or specialisation is that people become experts in their particular professions through constant practice. But in order that the society may exist as a coordinated unit, the various experts must enter into fruitful exchanges with each other for the common good of all. And there must also be means of control and communication, as indeed there are.

A similar specialisation or division of labour can be visualised in a complex organism. Indeed, the specialisation in the body has far exceeded that in the society e.g., the function of a cell can be identified by looking at it under the microscope ; a cell cannot change its function ; and when a cell divides it gives to cells that continue to perform the same function. Some fundamental activities (e.g. uptake of nutrients, excretion of waste products) are common to all cells but there is a specialisation of one or more basic function. There are cells whose function it is to cause movement, others produce secretions, some have absorptive functions, and still others transmit activity rapidly and over long distances. Now, in order that the body may react as a unit, there must be means of communication and control to coordinate the activities of various systems. Two such systems—the *nervous* and the *endocrine*.

rine—perform this function. The nervous system is a rapidly-acting system and controls activities such as movement, visceral functions, and secretion of some endocrine glands. The endocrine system, on the other hand, is a slowly acting system and controls metabolic activities. Both systems are involved in reproduction and some other functions.

A *control* or *homeostatic* system is a collection of interconnected components that act together to keep a chemical or physical parameter (e.g., blood glucose, blood pressure, temperature) of the body relatively constant. In a system *inputs* are those stimuli or disturbances that act on it, while the *output* or response is the result of such inputs. The constant value being regulated by a system is called its *setpoint*. Most control systems function on the principle of *negative feedback*. This is the process of control in which the response of the system is opposite (i.e., negative) to the initiating stimulus. For example, if the blood pressure falls due to any reason, the control system brings about a reflex rise and vice versa. There are literally thousands of control systems in the body. Some act at molecular, cellular and organ system level while others act at individual behaviour and social levels of organisation.

For any physiological system, three basic questions are relevant : (1) what is happening ? (2) what is the rate of its happening ? and (3) what determines (regulates) events and sets the rate ? These questions may be asked at any level of organisation.

Experimentation and Observation

Experimentation is a time-honoured procedure in the process of gaining knowledge about any science. An experiment consists in making an event occur under certain known conditions, care being taken to eliminate as many extraneous influences as possible. It is very important to consider the essential points of a technique during experimentation. Such care bestowed on apparently small, seemingly unimportant, yet troublesome points, usually determines the outcome of the experiment. It must be remembered that mistakes in technique are reflected in misleading results. For these reasons, the student must devote full attention and care to the procedure of an experiment.

The student must also inculcate the habit of observation right from the beginning. Relationships between phenomena can only be revealed if proper observations have been made. Observation should not be passive. Active and effective observation involves noticing something, and giving it significance by correlating it with something else noticed or already known. In this particular sense observation contains both an element of sense preception and a mental element.

A. N. Whitehead says, "First-hand knowledge is the ultimate basis of intellectual life. The peculiar merit of scientific education should be, that it bases thought upon first-hand observation, and the corresponding merit of a technical education is that it follows our deep natural instinct to transfer thought into manual skill, and manual activity into thought. The thought which science evokes is logical thought."

Students have a common tendency to report observations similar to those described in the books. One should always remember that the results are, strictly speaking, valid only for the precise conditions under which the experiment was conducted. It is

also a well known fact that the accuracy with which an experiment is conducted varies from individual to individual as does the accuracy of observation. However, the fact that the students must realize the importance of making careful observations on their own experiments, and report these as such, does not necessarily mean that the results must differ from those given in the books. If the results are at variance, some unrecognised factor or factors may be operating. Such occurrences should be welcomed because a search for an unknown factor may lead to some interesting discovery. It is when experiments go wrong that we find things out.

INSTRUCTIONS TO THE STUDENTS AND LABORATORY DISCIPLINE

During the course of training in practical physiology the student will come across a wide variety of apparatus and instruments. It is essential that a student check out his apparatus before starting the experiment. It should be in proper working condition so that his attention is not distracted by any faults in it ; this will enable him to concentrate fully on the biological phenomena under study. It is vital for the student to pay due attention to the practical demonstration given by the teacher before every experiment. It would be of further help if he consults the book beforehand to plan and organise each experiment. The experiments are so designed as to lead the student through the use of each instrument, from Amphibian to Human experiments in a logical fashion. The student should not proceed if he cannot answer questions and is not satisfied with his experiment ; help from a teacher must be sought, if needed. It is important that each student understand the use of every apparatus and be able to conduct the experiment by himself. As the students will be working in groups, one should not expect, nor depend entirely on the efforts of other members of the group to do most of the work.

It is not necessary to write up a very detailed account of the procedure of every experiment. In some experiments, e.g., the 'simple muscle twitch'—the first experiment in 'nerve-muscle

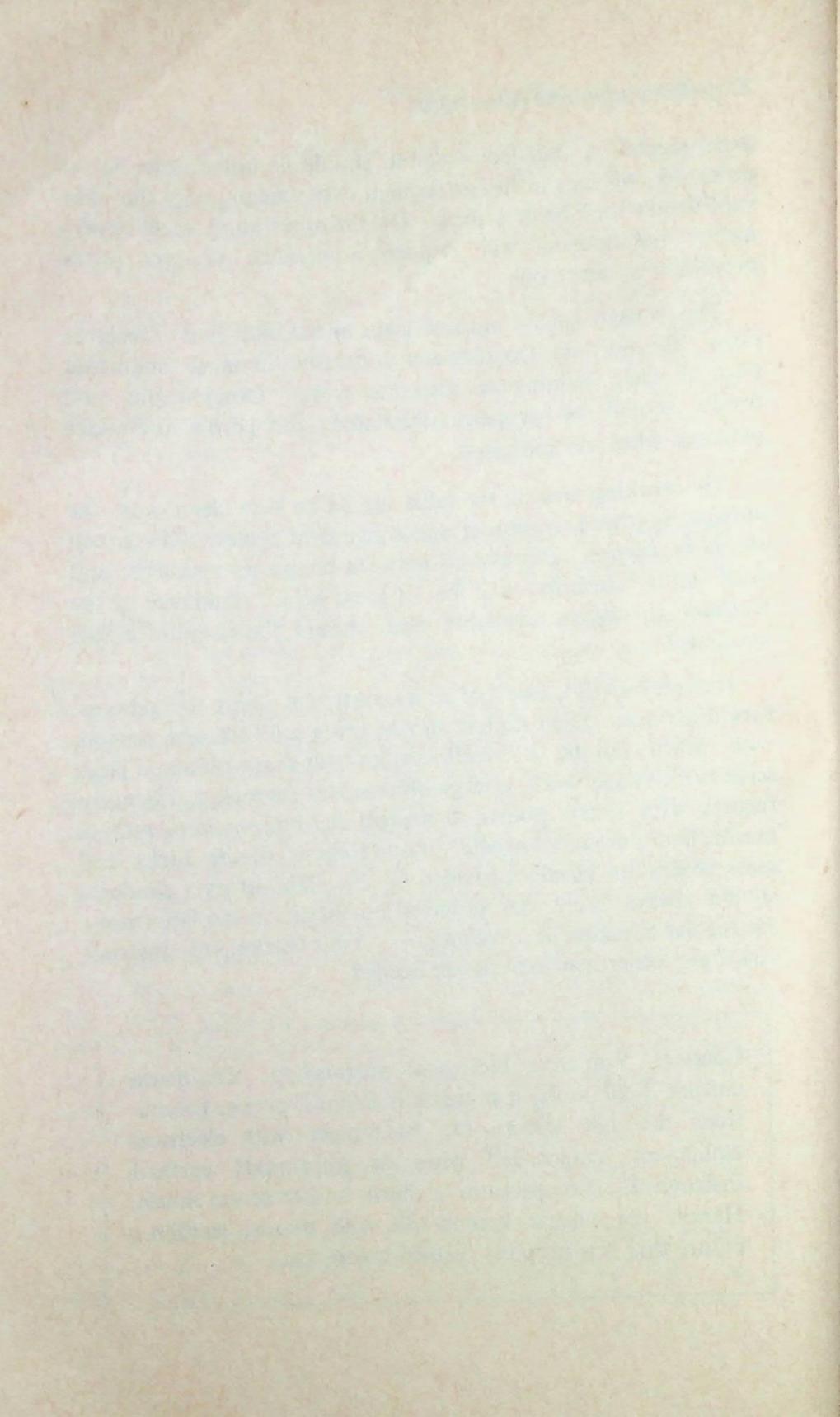
'experiments', a detailed account should be noted down in the notebook, whereas in the subsequent experiments, only the relevant details need be described. On the other hand, most experiments, on humans will require a detailed account of the procedure or technique.

The students should not lose sight of the fact that 'Observations', 'Results' and 'Conclusions' constitute the most important part of their training in scientific work. Observations and results should be properly tabulated, and graphs and tables prepared whenever indicated.

The working area on the table should be kept clean and the apparatus placed in proper and convenient places. *Idle gossip should be avoided.* Discussions between the group members and with other students will be of great help. Guidance by the teachers is always available and should be sought for and welcomed.

A clean overall is essential as it constitutes a part of laboratory discipline. Each student should bring a 6" straight scissors with sharp points, a small scissors with sharp points, a large scissors for rough work, a large blunt-nosed forceps, a dissection forceps with sharp points, a straight and sharp-pointed pithing needle, and a mounted needle. Bone forceps, tuning forks and glass probes are usually provided by the laboratory. Students should always bring the practical notebooks in the laboratory for regular checking and evaluation. Your teacher will instruct you if any other instruments are needed.

Caution. You are a biological preparation. The mains current (220 volts) is potentially lethal if proper precautions are not taken. Do *not* tamper with electrical equipment; uninsulated wires or improperly earthed instruments can produce a burn and/or severe shock. Handle the electric kymograph with utmost caution; ensure that it is properly earthed before use.



SECTION I

AMPHIBIAN EXPERIMENTS

Introduction

The student usually begins his experimental work on frog's nerve and muscle tissues. The great advantage of these tissues is that they can function without blood supply when kept in a proper solution or when the solution is poured on them. Ringer's solution is ideal for this purpose. The tissues get their oxygen by diffusion from the outside air. The frog's Gastrocnemius muscle—sciatic nerve preparation, first employed by Jan Swammerdam in the later half of 17th century, is a simple preparation, convenient to handle and to record the biological phenomena under study. This preparation is easily available for experimental work for 2-4 hours.

The frog gastrocnemius muscle has a much larger cross-sectional area than sartorius, hence develops the much greater force necessary for moving the isotonic lever for recording the isotonic contractions. In the experiments that the student will be performing, the muscle contracts isotonically.

This section includes some simple experiments whose completion requires little theoretical knowledge, and marks the beginning of the laboratory work that is concerned with specific properties of living tissues.

Experiment on Nerve and Muscle

Experiment No. : 1.1 STUDY OF APPARATUS

A brief description of the various instruments to be used in routine laboratory experiments is given below :

1. **Source of current.** In order to get an induced-current stimulus, a constant current (Galvanic) of low voltage is fed into the primary coil of the induction coil. For the supply of this low-voltage direct current, a *central low-voltage unit* is installed in most laboratories. This apparatus combines a step-down transformer with a rectifier unit and is plugged in direct to the mains supply (AC 220 volts, 50 Hz). The output terminals feed 2-15 volts *direct* current to all the experimental work seats where induction coils can be conveniently connected.
2. **Simple key.** This is included in the primary circuit between the low-volts output and the induction coil. Many types are available, e.g., metal blocks, morse, switch type and the unspillable mercury key.
3. **Induction coil.** (Du-Bois Reymond inductorium). It was introduced by Du-Bois Reymond in 1849 and is a simple device for transforming the *direct* (Galvanic) current into an exciting form—the Faradic or *induced* current. Its chief merit is the simplicity of operation, hence its use for routine experimental work.

In order to understand the working of the induction coil, it is necessary to understand the principle on which it is constructed. Consider two metal wires *P* (primary) and *S* (secondary) placed near to and parallel with each other (Fig. 1.1). Constant current can be made to flow through the wire *P* and it can be switched on or off with a simple key. When the primary circuit is 'made' or 'broken' by closing or opening the simple key, *induced* current is set up in the wire *S* as shown in the Fig. 1.1. During the continuous flow of current through the wire *P* there is no *induced* current. When the current is switched off in the wire *P*, there is *induced* current in the wire *S*. Thus *induced current is obtained only at 'make' or 'break' of the primary circuit.* Furthermore, any sudden change in the strength of current in the primary circuit will also produce *induced* current.

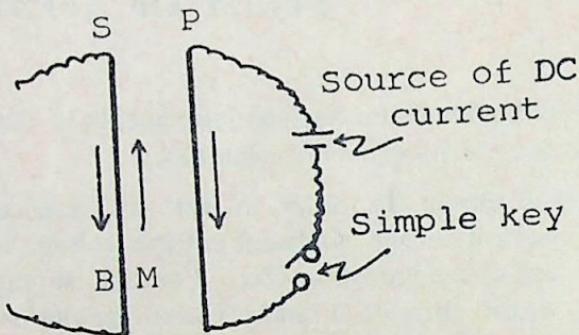


Fig. 1.1 Genesis of induced current

At 'make' the flow of *induced* current in the wire *S* is in a direction opposite to that in the wire *P*. At 'break' the *induced* current is in the same direction. The *induced* current has two important features viz., short duration, and a high electromotive force.

In order to multiply the induction effects, the two wires, *P* and *S*, are replaced by closely-coiled wires in the induction coil—the primary and the secondary coils. (Fig. 1.2). The primary coil consists of 250 to 300 turns of relatively thick, cotton-covered copper wire, wound on a wooden reel which contains a bundle of soft iron wires in its core. These wires, by their magnetisation enhance the induction effects. The two ends of the wire are connected to two terminals mounted on the induction coil. The secondary coil has 7000 to 8000 turns of thin, enamelled, copper wire

wound round a hollow wooden reel. The two ends of this wire are also connected to terminals mounted on the secondary coil, which moves in a groove in the base of the apparatus, and can be made to slide towards or away from the primary. The distance between the two coils determines the strength of the *induced* current—the greater the distance, weaker the *induced* current.

In some induction coils, the secondary can be slid off the primary to a distance of 15 cm, and at this point, further reduction in the strength of *induced* current is obtained by rotating the coil horizontally up to an angle of 90° to the primary coil, the angle being indicated on a calibrated scale. The following factors determine the strength of *induced* current :

- (1) The number of turns in the two coils—this number is of course fixed.
- (2) The distance between the two coils—this can be altered.
- (3) The strength of the *direct* current fed into the primary coil—it can be increased or decreased.

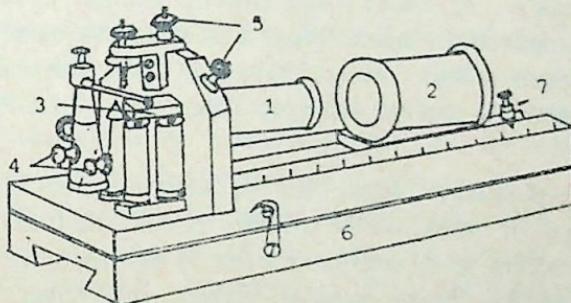


Fig. 1.2 The Du Bois-Reymond induction coil. 1—primary coil ; 2—secondary coil ; 3—Neef's hammer assembly ; 4—terminals for Neef's hammer ; 5—input terminals for primary coil (the output terminals of the secondary coil are not shown) ; 6—wooden base with scale ; 7—pointer, for indicating distance between primary and secondary coils

- (4) The angle between the coils—if the secondary coil is placed at right angle to the primary, there is no *induced* current.

For obtaining single 'make' or 'break' induction shocks, the primary circuit is closed or opened with the simple key. When repeated and multiple induction shocks are required, a Neef's

hammer, fitted to the side of the induction coil, is used. It automatically and repeatedly 'makes' and 'breaks' the primary circuit. It consists of an electromagnet and a horizontally mounted T-shaped iron bar with a spring. When the Neef's hammer is included in the primary circuit and the current is switched on, the electromagnet gets magnetised and the iron bar is drawn down. The circuit now being broken the electromagnet is no longer active, the iron bar springs up and establishes contact with a screw. This completes the circuit again and the magnet pulls down the iron bar once more. Thus the spring vibrates to and fro, and 'make' and 'break' *induced* currents are set up in the secondary coil many times (60-70 or more) per second. Platinum points are provided on the iron bar and the contact screw to withstand sparking.

'Break-induced current v/s 'make' induced current. The strength of 'make' and 'break' *induced* current depends on the rate of change of current in the primary circuit, whether this change is from zero to a certain level (at 'make'), or from that level to zero (at 'break'). At 'make', the rate of change of current is slower as compared to 'break' because of the extra currents that are self-induced within the primary coil. For this reason, the 'break'-induced current is stronger than the 'make'-induced current.

It may be pointed out again that the *induced* current is obtained only at 'make' or break' of the primary circuit and that there is no *induced* current while *constant* current is flowing in the primary circuit. Also, the 'break'-induced current is stronger than the 'make'-induced current.

4. Short-circuiting key. It is a useful device for the prevention of accidental passage of *induced* current into the tissues under study. It also prevents unipolar induction.

Various types are available viz Du-Bois Reymond, Sherrington, and the switch type. In the former two types there are two metal blocks, each with two terminals, mounted on a wooden or a vulcanite base. A metal clip bearing a handle is fixed to one block; when the handle is pushed down it connects the two metal blocks. In the switch type, there are four terminals and a switch mounted on a base. The two right-hand terminals are

permanently connected as are the two left-hand terminals. The two poles of the switch are connected to the two inner (or the two outer) terminals. Instead of leading two wires from the secondary coil directly to the stimulating electrodes, a short-circuiting key is interposed in the secondary circuit (Fig. 1·5). The two wires from the secondary coil are connected either to the two outer terminals or to the two inner terminals and connecting wires are led to the stimulating electrodes from the other terminals.

When the switch is put on the on position, a short circuit occurs and the *induced* current, taking the path of least resistance, passes back into the induction coil. Pushing the handle down in the other types of keys will cause a short circuit in the same manner by connecting the two metal blocks. Only when one desires to stimulate a tissue, the short-circuiting key is opened i.e., put on the off position, (so that the induced current may pass into the stimulating electrodes) otherwise it is kept closed i.e., put on the on position.

5. Stimulating electrodes. These are employed for delivering the electrical stimuli to the tissues. The common variety supplied with the frog muscle chamber consists of ball and socket-mounted-silver electrodes fitted to a clamp bearing two terminals which can be screwed along one side of the chamber. These electrodes can be easily manipulated into the required position. Wooden electrodes are quite simple to handle and are used in some experiments. Two small copper wires are fitted into a thumb-sized wooden (or vulcanite) block. Other electrodes for student's use include bipolar platinum (stout, sheathed copper wires with connectors and ending in platinum points) and zinc electrodes.

Specially constructed electrodes are used for work in electrophysiology. Some of these are : Unipolar platinum, concentric needle (for EMG etc) and glass-capillary microelectrodes ; the last are precision made and have a tip diameter of about one micron (for intracellular recordings). Besides stimulating electrodes, recording electrodes are necessarily employed in such studies.

6. Frog board and muscle chamber (muscle trough). The frog board myograph has a wooden board ($6'' \times 4'' \times 1''$) with a $1/4$ inch cork top, for keeping the muscle in position while its contraction is being recorded by the Starling myograph lever fixed to the board. One disadvantage of frog board is that the preparation needs frequent pourings of Ringer to prevent its drying. A modified frog board has sides raised to $1/4$ inch for holding saline.

The frog muscle chamber is a transparent perspex bath ($6'' \times 4'' \times 2''$), with a clamp and drain pipe, in which the muscle can be completely immersed in the Ringer solution. Two full-depth corks fitted in the bottom of the chamber take pins for fixing the muscle. The trough is carried on an upright stand of standard size (QTVT stand) which has a quick-stop arrangement and micrometric adjustment at its base for turning the rod on its axis. The 'stop' prevents the writing point from pressing too hard against the paper. When used in this way, the point can be removed at any time from the paper, and then, brought back again so as to press with exactly the same force as before. It is essential to make use of the 'stop' in all recording experiments in which comparisons of different curves upon the same surface have to be made. Failure to observe this precaution will

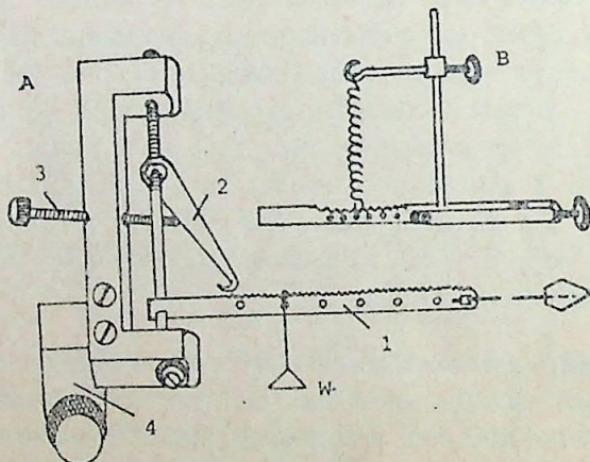


Fig. 1.3 (A) The writing lever(isotonic) used for recording muscle contractions. 1—horizontal arm; 2—vertical arm; 3—after-load screw; 4—assembly for fitting the lever on the muscle chamber.

(B) Starling heart lever

invalidate the results. Ordinary adjustable stands can also be used, provided necessary precautions are taken.

7. **The writing lever.** (Fig 1.3) It is employed for magnifying and recording the activity of a tissue manifesting movement. The L-shaped lever as shown in the figure, is used for recording isotonic contractions. It consists of a *horizontal arm* which bears holes and notches for hanging a weight, and a *vertical arm* which bears a hook at its end and which descends into the muscle chamber ; the tendon of the gastrocnemius muscle is tied to the hook through a thread. The two arms must remain firmly fixed to the spindle to remain at right angles to each other to give maximum leverage and magnification during the recording of contraction. The horizontal arm carries a light-weight capillary lever with a writing point at its end. Ink writing levers are also available.

The after-load screw, fitted in the frame of the lever assembly, (Fig. 1.3) is shown supporting the vertical arm of the lever ; contractions recorded in this position will be after-loaded ones (the load or weight acting on the muscle after it starts contracting. All contractions recorded in the following experiments, except a few in experiment 1.13., will be after-loaded ones. When the after-load screw is pulled back so that it no longer supports the vertical arm of the lever, the contractions recorded would be free-loaded ones—the load or weight acting on the muscle before it starts contracting—and stretching it.

8. **Starling's heart lever.** (Fig. 1.3.) It is employed for recording the mechanical events of the frog's heart. The frame fits over a standard rod and carries a light flat lever arm with a finely adjustable tension spring which supports the writing lever in the horizontal position. A piece of thread tied to the lever carries a bent pin at its free end and the pin is hooked through the apex of the ventricle. When the heart contracts it pulls the lever down and when it relaxes the spring pulls the lever back to the horizontal position.

9. **Isometric lever.** It consists of a holder carrying a steel tension spring and a flat lever with holes, and is used for recording isometric contractions of the muscle.

10. Vibrating reed. It is hand operated and calibrated to give 5, 7, 10, 15, 30 vibrations/sec, and is mounted on a wooden or vulcanite base. It is provided with mercury contacts and an occasional tap with a finger maintains vibrations over a long period. This apparatus is used where rapid 'make-break' induction shocks are required (the reed is included in series in the primary circuit). Electrically operated vibrating reeds are also available.

11. Pohl's commutator. This is used for changing the direction of current. There is a vulcanite base on which a rocking metallic cradle is mounted. There are six cup-like depressions filled with mercury and six terminals are attached to these ; two narrow copper strips connect the diagonally opposite corner cups.

12. Rheocord. About 2-3 metres of resistance wire is mounted on a wooden board and its ends are connected to two terminals. A metal contact block, bearing a terminal is provided and can be placed at any point of the wire.

13. Variable interrupter. It works on the same principle as the Neef's hammer. It has an electromagnetically operated pendulum with a platinum contact point and a stop screw that controls its arc of swing. There is a spring which also bears a platinum contact point. By manipulating the stop screw the arc of swing of the pendulum can be altered and a variable number (6-80/sec) of induction shocks can be obtained by including it, in series, in the primary circuit.

14. Time marker. (a) An electromagnetic time marker is used in conjunction with the central low-voltage unit and can provide 'make-break' contacts at time intervals of 1, 2, 5 and 10 seconds. It is connected directly to the 'timer' terminals (which are connected to a central timing unit) on the work table. It can be used as a signal marker for recording an event by including it in the primary circuit, as will be explained later. (b) *Spring time marker.* It is a heavily weighted and calibrated vibrating spring to which a writing lever can be fixed to inscribe directly on the smoked paper. A light and occasional pressure of the finger will maintain its vibrations. Usually, two adjustments of $1/2$ sec and $1/5$ sec per complete cycle are provided,

thus half waves of $1/4$ second and $1/10$ second can also be inscribed. (c) *Tuning forks.* Tuning forks of 100 and 256 cycles per second ($n=100$, $n=256$) with writing stylus, are used as time markers in nerve-muscle experiments for determining the durations of latent, contraction, and relaxation periods. Electrically operated tuning forks can also be used, the vibrations being electromagnetically controlled and maintained.

15. **The Kymograph.** It is a machine for obtaining and displaying graphically, the time course of events in the tissues manifesting movement.

It consists of a cylindrical drum (the 'cylinder') and a mechanism for rotating it at various speeds (different speeds are provided as the speed of events being recorded varies in different types of experiments). The cylinder is covered with a highly glazed and 'smoked' (or 'unsmoked') sheet of paper on which the record is traced by a suitable writing lever.

Clock-work, mechanical (pulley driven) or electrical kymographs of various designs for routine and research work are available. Although the proper term for this apparatus is 'kymograph', the term 'drum' (which is commonly used both for the machine as well as the 'cylinder') is too firmly entrenched to be replaced easily.

Electrical Kymographs are preferred for more accurate work and are available in most laboratories for students' use. These are easy to operate and the basic working of various designs is identical. The teacher in charge of the laboratory will explain the working of a particular type of kymograph. However, the "Palmer type" electric kemograph is taken here to explain the working of this apparatus (Fig. 1.4). Its components are :
(a) *Electric motor.* Constant-speed, governed motor operating on AC mains 220 V 50 Hz, is enclosed within the kymograph body.
(b) *On/off switch* for mains. (c) *Gear system* It is connected to the electric motor and provides different speeds for the recording surface. (d) *Variable speed setting lever.* It moves horizontally in a slot marked F (fast) and S (slow) at its ends.
(e) *Calibrated speed setting.* This is located on the side of the machine. The two prongs of the lever have to be pressed

together before it can be moved up or down for engagement at a particular gear. There are five notches or gear positions where the gear lever may be engaged, one of these being the 'neutral' position (second from the lowest notch). The four engagements of the gear lever at a particular 'calibrated setting' give the following speeds (slow-fast) : 0.12/0.25—0.12 mm/sec and 0.25 mm/sec ; 1.2/2.5—1.2 mm/sec and 2.5 mm/sec ; 12/25—12 mm/sec and 25 mm/sec. 'N' is the neutral position;

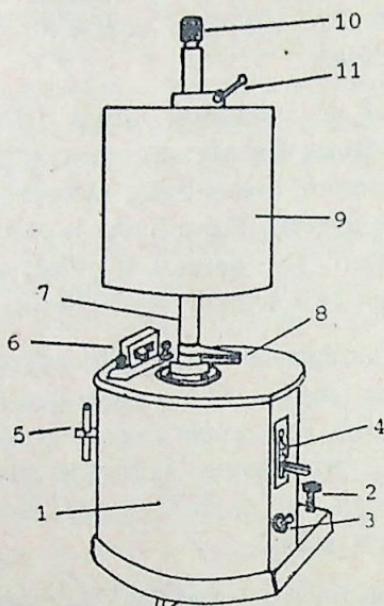


Fig. 1.4 Electric kymograph. 1—Kymograph body containing electric motor and gear system ; 2—Levelling screw ; 3—on-off switch for mains current ; 4—calibrated speed setting system ; 5—clutch lever ; 6—electric contact block ; 7—spindle ; 8—dual electric contact arms ; 9—cylinder ; 10—screw lift for cylinder ; 11—lever for fixing cylinder on the spindle. [The variable speed setting lever is not visible in this view].

320/640—320 mm/sec and 640 mm/sec. The calibrated setting lever must not be changed while the cylinder is rotating as it is likely to damage the gear system. Whenever one wishes to rotate the cylinder manually, either the setting lever must be engaged in the notch marked *N* or the clutch lever, which engages or disengages the gears, should be used. Failure to

observe these precautions will damage the gears. (f) *Clutch lever.* This engages or disengages the gears when it is placed in vertical or horizontal position.

How to express the speed of the kymograph. The speed is *Not expressed in revolutions* but as "millimeters passing across a point of contact on surface of a 6 inch diameter cylinder. For example, if the setting lever is engaged at the lowest notch (640/320 mm/sec) and the 'slow-fast' variable speed setting lever is moved towards the *F* (fast) position, 640 millimeters will pass across the writing lever point every second. (In research and long paper extension kymographs, speed is expressed in the same manner i.e., in mm/sec). It may be noted that by adjusting the variable speed setting lever anywhere between *F* and *S*, innumerable, though not exact, speeds are possible, but this is, however, rarely required. (g) *Spindle.* It projects up from the top of the kymograph and is connected to the gear system in the body of the apparatus. A screw-lift arrangement is provided at its upper end to raise or lower the cylinder. Dual electric fixed contact arms (also called the 'striker') are fitted to its lower end. These are used for marking the point of stimulation on the surface of the recording paper. (h) *Cylinder.* A $6'' \times 6''$ cylinder is the standard size and has a notch to help guide it slip over the spindle. It can be fixed firmly on the spindle at any level with a locking lever provided at its top. (A loosely-fixed cylinder moves on the spindle and invalidates the experiment). A glazed paper sheet is wrapped around the cylinder, evenly and smoothly, with the glazed surface outside and fixed in position with gum or adhesive cellophane tape in such a way that the seam or overlap thus created will not catch the writing point. (i) *The contact block.* It is mounted on the kymograph at the level of the 'striker' and has a 'spring-contact' and two electrical-contact terminals, both with platinum contact points. The contact block as a whole can be adjusted and fixed firmly in the correct position by a screw. When the contact block terminals are included in the primary circuit, the circuit will be completed only when the striker touches the metal spring and brings the platinum points into contact with each other. The contact block terminals thus act as a simple key in the primary circuit but its purpose is to mark the point of stimulation on the paper. As the spindle is rotating at

a fast speed (640 mm/sec), there is an instantaneous 'make' followed immediately by 'break' of the primary circuit with each revolution of the spindle. The two induction shocks thus produced, act as a single stimulus, since, the second shock (at 'break') falls within the refractory period of the first induction shock (at make). Thus, *it is the 'make' induced current which is effective stimulus* for the nerve or muscle. With slower speeds, the 'make' shock can be eliminated by moving the secondary coil to a distance at which it becomes ineffective. (j) Two levelling screws are provided at the base for adjusting the tilt of the kymograph on the work table.

There are other types of kymographs in use in different laboratories. Your tutor will explain the working of a particular variety.

Note. When the preparation is in contact with the electrodes it can be stimulated accidentally when touched by the student, or by instruments held by the student. This is apparently due to A.C. pick-up in the student causing sufficient operator-to-nerve current to stimulate the nerve. The student should watch out for such stimulation, which can be avoided by ensuring that the student is earthed (e.g., by touching the body of the kymograph, which is already earthed) whenever he must handle the preparation.

16. **Smoking.** The cylinder is slipped over the horizontal spindle of the smoking stand and the glazed paper is 'smoked' with an intensely black and smoky flame obtained by passing coal gas through benzene in the handle of the burner. It is important that the layer of soot should be thin and uniformly black. The paper will get burnt if it does not fit evenly and tightly around the cylinder or if the flame is played on a stationary cylinder.

17. **Varnishing.** A 2 per cent solution of shellac or resin in methylated spirit is used as a varnishing and fixing medium. After obtaining and labelling the record, the paper is cut through the overlapping part with a scissors (do not use a scalpel as it will mark the cylinder surface) and passed through the fixing solution placed in an enamel tray. When the paper dries, a fine coat

of shellac or resin remains on it, thus giving a permanent record. This is then pasted on the blank page of the experimental note-book.

18. Student stimulator. Transistorised or electronic stimulators with a D.C. output ranging from 0-15 volts are now available. Each main stimulus parameter (volts, frequency and duration of the stimulus) is indicated on the apparatus. The following knobs and selector switches are provided :

(a) *On/Off switch.* A.C. mains 220 V 50 Hz (b) *Output voltage control.* (c) *Mode of operation switch.* single, repetitive or an external trigger. The external trigger is for use with the electrical contacts of the contact block ; each time the fixed contact on the spindle strikes the contact-maker, it short-circuits the EXT. TRIG. terminals and initiates a pulse. (d) *Pulse frequency knob.* For selecting the desired rate of repeated stimulation (1-100/sec). (e) *Pulse duration.* 0.5 or 1.5/sec. (f) *Terminals for output* and (g) *Terminals for trigger circuit.* More advanced stimulators ('Grass' stimulator) include extra circuits which are essential for certain types of experiments and research work.

19. Transducers. These devices convert some physical measurement, like force, pressure, displacement, temperature etc. into electrical signals which can be recorded on a CRO.

20. Cathode-ray oscilloscope (CRO.) It is designed to measure the electrical events in the tissues. Its component parts are : (a) *Electron gun.* This is the source of electrons emitted from a cathode, and which are converted into a beam by a pair of strong magnets. (b) *Fluorescent surface.* The rear surface of the CRO screen is coated with a fluorescent material. The electron beam falls on it and leaves a trace. (c) *Sweep generator.* Application of a saw-tooth voltage to metal plates, placed on either side of the electron beam, causes it to move steadily towards the positive plate (from left to right on the screen) and then to snap back to the previous position ; a straight line is thus inscribed due to very fast sweeps of the beam. (d) *Horizontal plates.* One is placed above and the other below the beam. Voltages applied to these, cause the beam to move up and down and since these plates can be charged positive or negative, the potentials from the tissues can be recorded after suitable magnifi-

cation. CROs are extensively used in hospitals (e.g. continuous monitoring of the electrical activity of heart in heart diseases) and in electronic industry besides many other fields.

ELECTRICAL CONNECTIONS

Fig. 1.5. illustrates the connections required for obtaining induced current stimuli.

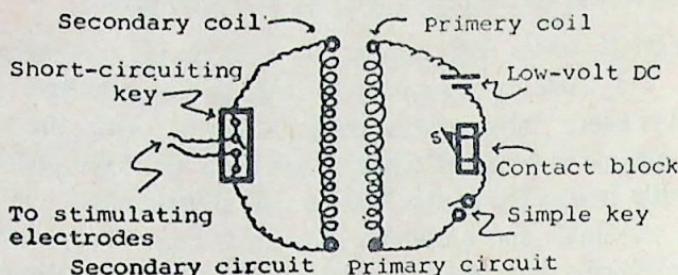


Fig. 1.5 Arrangement of the circuit for electrical stimulation of the nerve (or muscle). The contact block 'makes' the primary circuit by the striker of the rotating drum. Contact can also be made by hand, if desired, by tapping the spring(s) of the contact block with a finger to complete the circuit at this point.

Low resistance cotton-covered copper wire is used for making connections.. Wind lengths of this wire around a pencil so as to get coiled-wire pieces. These can be stretched as required and this prevents confusion in the working area. Remove the cotton insulation and bend the last inch of the wire in the form of a wide U. Unscrew the terminal, place the loop of the wire under it and tighten the screw so that there is a good metal-to-metal contact. (Do not wind the wire around a terminal as it becomes difficult to disconnect the wires at the end of the experiment.)

ARRANGING THE APPARATUS

Place the induction coil just in front of you on the work table and beyond this, kymograph. Clamp the simple key to the table on your right-hand side and the short-circuiting key to the left of the working area. The muscle chamber should be placed to the left of the induction coil so that the writing lever is at a tangent to the recording surface (you should be able

to watch the writing point of the lever without unnecessary bending and twisting).

Trouble-shooting. Even after making correct connections, there may not be any response after the application of a stimulus. You must learn to locate the fault by 'step checking' of connections and apparatus, starting with the primary circuit. (Confirm that there are no loose connections).

A. Primary circuit. Connect a short piece of wire to one of the low-volts terminals and strike the other terminal with its free end ; a spark indicates the presence of current. Check the simple key and the contact block on the kymograph. Each time the striker makes contact with the spring contact of the contact block a spark is produced ; sparking at the platinum contacts checks out the primary circuit.

B. Secondary circuit. Place the 'severed leg' from the frog on the wires connected to the secondary coil terminals, and if this twitches with each revolution of the spindle, the fault lies beyond this point. Put the short-circuiting key on the 'off' (open) position, and place the severed leg on the electrodes. Switch on the kymograph, if there is a response, the induction shocks are, evidently, reaching the electrodes. If the muscle is not contracting through stimulation of the nerve, place the electrodes directly on the muscle and if the muscle contracts, the nerve has obviously been damaged during dissection. Replace this preparation with the one kept for such contingency. If all efforts fail, seek the help of the electrician and your tutor.

Composition of Ringer's Solution (Ringer)

Sodium chloride-0.6 g ; calcium chloride-0.01 g ; potassium chloride-0.0075 g ; sodium bicarbonate-0.01 g ; distilled water to 100 ml.

Note The Ringer fluid is isotonic with frog's tissues and has optimal ionic constituents Isotonic saline for frog is 0.6 per cent NaCl solution. The isotonic for mammals is 0.9 percent NaCl solution.

TERMS USED

Excitability. This is the faculty or property of living tissues reacting to changes in the environment. Each tissue reacts to

such changes in its own particular fashion. A muscle fibre responds by contracting, a gland cell by elaborating a secretion and a neurone by transmitting a nerve impulse. Other cells respond in the form of chemical changes. In each case there is a change in the metabolism of the cell. If the change in the environment (stimulus) is gradual, the tissue does not respond to it, it gets adapted to the change. In order to be effective, therefore, the stimulus must reach the threshold level within a very short time. It may be noted that the response of a tissue is not always of an excitatory nature, the activity can be inhibited as well.

2. Stimulus. A stimulus is a change in the environment of an excitable tissue which causes the tissue to respond in its own particular manner.

A. Types of stimuli. The different types of stimuli are : (1) Physical (drying, cooling, warming), (2) Mechanical (tapping or pinching), (3) Chemical and (4) Electrical. The electrical stimuli are employed most commonly. Their strength and duration can be easily controlled and they do not cause any damage to the tissues as their duration is very short. Furthermore, they resemble the natural mode of stimulation of the tissues and can be employed repeatedly. The various forms of electrical stimuli used for stimulating conductive tissue are :

(a) *Induction stimulus.* It is obtained from an induction coil. The break induction shock is of opposite polarity, higher voltage, and of a shorter duration as compared to the make stimulus. The students will be using this form of electrical stimulus in their experiments on amphibian preparations. (b) *Sine wave stimulus.* The ordinary house-hold current, the sine wave form, 220 volts AC at 50 Hz (cycles/sec) is not desirable as it lasts much longer than the one thousandth of a second or less needed to stimulate a tissue. This stimulus may actually burn the tissues. (c) *Rectilinear (rectangular) stimulus.* An electronic apparatus is needed to provide such stimuli. The voltage rises to a desired and preselected level immediately and can be maintained at that level for the required duration. Thus, the strength in volts and duration in milliseconds can be selected. This is the most common form of stimulus employed in neurophysiology.

gical work. (d) *Condenser discharge*. A condenser is charged to high voltage and this current is then applied to the tissue. The current rises to a certain level and then reaches zero gradually. (e) *Thyatron discharge*. The current from a charged condenser can be applied through a thyatron tube. The gradual decline of current in this case is cut off. The duration of condenser and thyatron discharge stimuli can also be controlled.

B. *Degree of stimuli*. The following degrees of stimuli, in terms of their strength are recognised :

(a) Subthreshold, subminimal or subliminal stimulus. These terms denote a stimulus which is not adequate to produce a response. (b) Threshold, minimal or liminal stimulus. This is the minimum stimulus just sufficient to produce a response. (c) Submaximal. (d) Maximal. (e) Supramaximal.

QUESTIONS : (1) What are the different types of stimuli ? Why are electrical stimuli preferred over other types ? (2) What type of current is fed into the primary circuit for obtaining induced current from the secondary coil ? (3) What are the factors that determine the strength of induced current ? (4) Why is 'break' induced current stronger than the 'make' induced current ? (5) Why is short-circuiting key included in the secondary circuit ? How is the speed of kymograph expressed ? (7) What is meant by excitability ? Which tissue is most excitable ? (8) How does a stimulus affect an excitable tissue ?

*Experiment : No. 1.2***DISSECTION OF GASTROCNEMIUS
MUSCLE-SCIATIC NERVE PREPARATION****DISSECTION STEPS**

Keep the preparation moist with Ringer during the dissection as well as afterwards.

1. **Stunning.** Hold the frog, gently but firmly by its waist, in a duster cloth. Give a good blow on the head with a wooden mallet ; only one or two blows should suffice to render the animal unconscious.

2. **Pithing.** Using a duster cloth, hold the animal in your hand, keeping its head flexed with the index finger. Push a pithing needle firmly through the skin, muscle and bone tissue into the spinal canal, at a point where a line joining the posterior borders of the tympanic membranes cuts the midline. Manipulate the needle anteriorly into the skull and destroy the brain by rotating it. Withdraw the needle and direct it backwards into the spinal canal to destroy the spinal cord in the same manner. As the spinal cord is being destroyed, the muscles of the limbs and trunk are thrown into contraction ; this is due to irritation of spinal motor neurons. When the spinal cord has been properly destroyed, the hind limbs will hang down limply and loosely.

This animal is now dead in the sense that it is no longer conscious and there are no voluntary or reflex movements of the limbs, but the various organs, (heart, nerves, muscles etc) are still 'alive' and can be used for physiological experiments.

3. Cut through the skin, with scissors, completely around the trunk just below the forelimbs. Seize the skin around the trunk in a piece of cloth and strip this 'trouser' of skin upto the toes of the hind limbs. Place the skin and other animal waste in the dissection tray.

4. Place the frog on its ventral surface. Pick up the tip of the urostyle with a forceps and give a cut under it with a scissors. Cut through the muscles attached to either side of it, taking care not to injure the nerves lying just underneath. Extend these lateral cuts forwards and cut through the hip gridle on either side with a bone forceps. As the urostyle is lifted, the sciatic nerves can be seen emerging from the vertebral column along with other nerves. Cut the vertebral column above and below the exit of the sciatic nerves. Do not attempt to separate the nerves at this stage.

5. Now you have a piece of vertebral column with sciatic nerves still attached to it. Divide this piece into two with a bone forceps by a slightly oblique cut. Lift up each piece and free the attached tissues. Snip away the nerve fibres going to the nearby structures, taking care not to injure the sciatic nerves which can be seen disappearing into the thigh muscles.

6. Cut through the fascia covering the thigh muscles and separate these with a blunt glass probe. The sciatic nerve will now be visible (Fig. 1.6). Holding the vertebral piece carefully with forceps, free the sciatic nerve to a point about half an inch above the knee joint. (As the branches of sciatic nerve to thigh muscle are snipped, the muscles show twitch-like contractions). (*Note.* Do not pick up the nerve directly with forceps

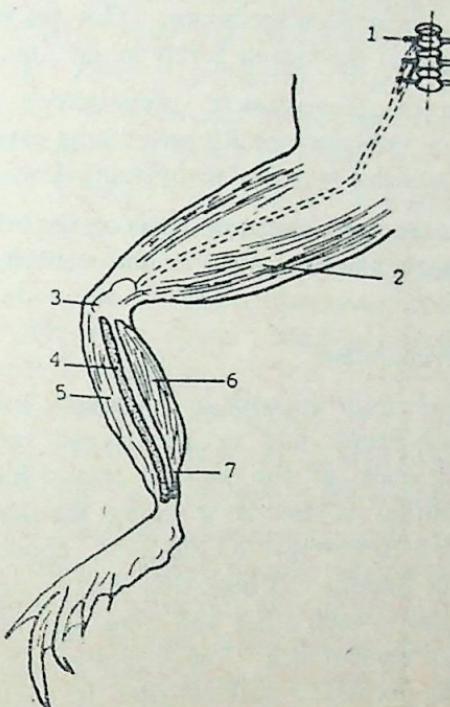


Fig. 1.6 : Dorsal view of the hind limb of the frog. The sciatic nerve is shown emerging from the vertebrae and coursing through the muscles of the thigh. 1—vertebrae ; 2—muscles of the thigh ; 3—knee joint ; 4—tibia ; 5—tibialis anterior ; 6—gastrocnemius muscle ; 7—tendo achillis.

but lift it by the attached piece of vertebral column. If once the nerve is damaged by mishandling, it is likely that the muscle will continue to twitch spontaneously, thus rendering the experiment impossible).

7. Separate the tendon of the gastrocnemius muscle from its insertion with a scissors and strip the muscle from the bone as far as the knee joint. Tie a stout thread tightly around the tendon just above the sesmoid bone.

8. Cut off the tibio-fibula below the knee joint with bone forceps and the thigh bone just above the point to which the nerve has been freed. Trim away any excess muscle tissue from the knee joint, taking care not to touch the nerve with scissors in the process. The dissection is now complete. Pass a pin through the knee joint, in between the bones. This pin will be required for fixing one end of the muscle firmly in the muscle trough.

9. Lift up the nerve-muscle preparation carefully and transfer it to a container of Ringer, taking care not to let the nerve (with its attached vertebral tissue) hang down in the process.

10. Repeat the same dissection steps on the other hind limb and place this preparation also in the container for possible use later.

Mounting the Preparation

Push the pin into the cork in the muscle trough to fix one end of the muscle firmly, and tie the thread (attached to the tendon) to the hook of the vertical arm of the writing lever. Adjust the position of the lever along the side of the muscle trough. Apply a 10 g weight on the lever, about an inch and a half from the fulcrum. Check that the two arms of the lever are at right angles to each other and that the after-load screw is supporting the vertical arm. The weight will bring the writing lever back to the horizontal position after the contraction is over.

Note that if the pin has been passed through the remnants of muscle tissue around the knee joint instead of through it, or if the pin is loosely fixed in the cork, the muscle will pull at both the ends when contracting and the recorded amplitude of contraction will be much reduced.

Experiment No. : 1.3

EXCITABILITY OF THE NERVE-MUSCLE PREPARATION

Make the following qualitative experiments and observations on the preparation, which may be kept on the frog dissection board or the tray. There is no provision nor an intent to record muscle contraction.

Observation 1. Mechanical excitation. A nerve or muscle can be stimulated by mechanical means. Pinch or tap the nerve with a forceps, the muscle gives a twitch-like contraction. It is due to the conduction of excitation from the nerve to the muscle. Pinch the muscle with a forceps, it responds by contracting, showing thereby that it is independently excitable.

Observation 2. Thermal excitation. Touch the nerve or muscle with a hot copper wire, the muscle shows contraction. Similar effects are obtained with a wire cooled to 0°C.

Observation 3. Osmotic excitation. Place a drop of glycerine upon the nerve, the nerve is stimulated as shown by muscle contraction ; a drop of strong salt solution produces the same effect. The stimulation in either case is caused by withdrawal of water from the nerve fibres. Wash the preparation with Ringer for the next experiment.

Observation 4. Chemical stimulation. Pour a drop of dilute acetic acid on the nerve, the muscle is seen to contract. The muscle is also independently excitable to dilute acids and alkalis.

Observation 5. Unipolar excitation. Include the interrupter in the primary circuit. Connect only one wire to one of the secondary coil terminals, place this wire on the nerve and switch on the current. The muscle shows contractions. To prevent unipolar induction, it is necessary, therefore, to use a short-circuiting key in the secondary circuit.

Observation 6. Excitation by galvanic current. Connect two wires to the source of galvanic current (low-voltage terminals), place a simple key in series with one wire, and connect the wires to the stimulating electrodes. Put the nerve on the electrodes and pass a make stimulus i.e., close the simple key. The muscle

contracts and then relaxes. If the current is permitted to flow continuously there is no response. Break the circuit i.e., switch off the simple key, there is another response by the muscle. If the electrodes are placed on the muscle, it also responds to both stimuli. It is important to remember that *the MAKE excitation starts at cathode, and BREAK at anode*. By using galvanic current of varying strength, it can be shown that muscle responds to weaker stimuli as compared to nerve.

Observation 7. Excitation by Faradic current. Arrange to stimulate the nerve and the muscle separately with 'make' and 'break' induction shocks of varying strength. You will find that the response appears first at break, and that the nerve reacts to a weaker stimulus than the muscle.

Observation 8. Excitation by drying. Allow the preparation to dry and as it is drying, the muscle begins to show irregular twitching. This effect is due to drying of the nerve and not the muscle which has a much greater bulk as compared to nerve. This type of twitching is a common source of confusion for the students ; it simply shows that the preparation has not been kept moist.

Conclusions. The observations demonstrate the independent excitability of nerve and muscle tissue to mechanical, thermal, chemical, electrical and physical stimuli. Observations 6 and 7 are relevant while testing for the reaction of degeneration.

Experiment No. 1.4 SIMPLE MUSCLE TWITCH

Principle. A single induction shock passed into the nerve results in a momentary twitch-like contraction of the muscle followed by relaxation. The upward movement of the writing lever is recorded in the form of a curve on the revolving cylinder. A time tracing is taken with a tuning fork for the calculation of various periods.

Apparatus and reagents. 1. Source of D.C. current. (central low voltage unit, accumulator or dry cells).
2. Simple and short-circuiting keys, and induction coil.
3. Kymograph, cylinder with smoked paper, frog muscle trough or board, low-resistance wire, stimulating electrodes, writing lever and weight hanger with 10 g weights.
4. Dissection instruments, pins, stout thread, tuning fork and dividers.
5. Ringer's solution.

Procedure. Read the description and working of the apparatus, electrical connections, dissecting and mounting the nerve-muscle preparation before starting the experiment. Check the primary and secondary circuits.

1. Arrange the apparatus in proper order in the working area and make connections for obtaining induced-current stimuli as shown in Fig. 1.5. Include the electrical contact terminals of the kymograph in the primary circuit.
2. Dissect a nerve-muscle preparation from a frog. Tie a piece of stout thread around the tendon proximal to the sesamoid bone embedded in it and transfer it to a frog muscle chamber or board. Tie the free end of the thread to the vertical arm of the writing lever. Adjust the positions of the cylinder and muscle trough so that the tracing is recorded about an inch above the lower edge of the cylinder.
3. Set the clutch knob in the horizontal position, engage the gear lever at the fastest speed and plug in the kymograph to the 220 A. C. supply on your work seat. Pick up the vertebral tissue with a forceps and place the nerve on the stimulating electrodes (the electrodes should not dip in the Ringer solution). Pass two or three induction shocks to verify a satisfactory amplitude of contraction; adjust if necessary. Switch off the kymograph and simple key and close the short-circuiting key. Put the gear lever on the neutral position. (Clutch lever is used for starting the kymograph).
4. Adjust the levelling screws of the kymograph and bring the writing point in gentle contact with the recording surface at a tangent. Rotate the cylinder with your hand so that a base line is inscribed all around the cylinder.

5. **Marking the point of stimulus:** Bring the striker in contact with the spring of the contact block so that the two platinum points are brought into contact ; a stimulus will pass at this position of the cylinder with every revolution of the cylinder. Keeping the striker in position with the left hand, raise the writing lever with a finger, above the base line. Check that it inscribes a line without much friction.

6. Switch on the kymograph and engage for the fastest speed. Release the clutch and let the speed of the cylinder become uniform. Switch on the simple key. The moment the striker just passes the contact-maker, open the short-circuiting key but close it again as soon as the muscle twitch is over. Stop the drum with the clutch lever, switch off the simple key, engage the gear lever at neutral position and move the lever away from the cylinder.

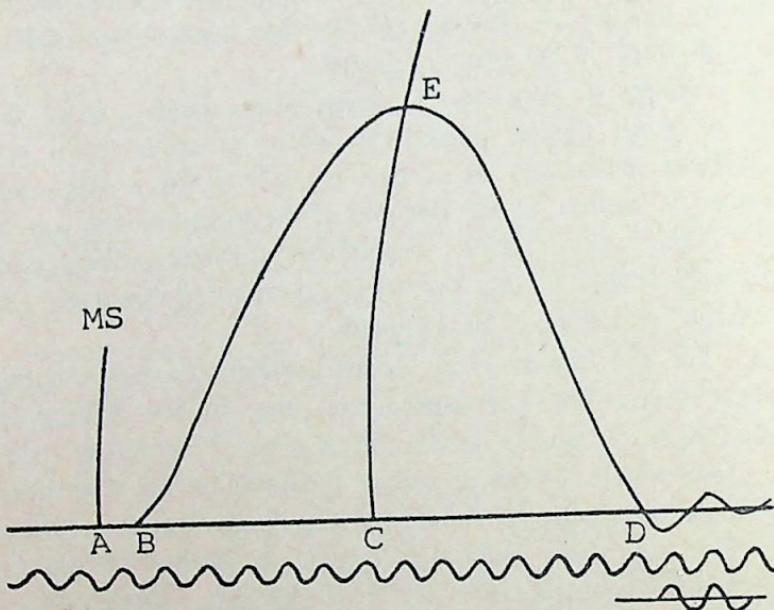


Fig. 1.7 : Simple muscle twich, its phases and time relationships. The waves at the end of the response are due to bouncing of the lever on the after-load screw and have no significance. MS-moment of stimulation ; AB-latent period ; BC-contraction period ; CD-relaxation period ; BE-contraction phase ; ED-relaxation phase. Tuning fork-100 Hz. Diagram of two waves of the time trace is shown below the time trace.

7. The interval between the point of stimulation and the rise of the curve is the *latent period*. Take the tip of the writing lever to the summit of the curve and draw a line in one continuous smooth movement from this point to the base line, dividing it into *contraction* and *relaxation* periods. Do not draw a vertical line from the summit to the baseline as the writing point does not move up vertically but in an arc-like fashion (Fig. 1.7.)

8. Recording the time-trace. Hold a tuning fork of known frequency ($n=100$ or 256) by the stem and set it vibrating by striking it against the palm of your left hand. Holding it horizontally, bring the vibrating stylus in contact with the paper *below* the baseline, moving it slightly downwards to prevent overlapping of the waves thus recorded.

9. Remove the cylinder from the spindle, discuss your graph with your teacher and get it signed before removing the paper from the cylinder. Cut across the overlapping part of the paper and remove it carefully without smudging it. Lay it flat, face upwards on your table and enter the following data *above* the tracing obtained.

- (a) Title of the experiment in capitals. (SIMPLE MUSCLE TWITCH)
- (b) Preparation used. (Frog's Gastrocnemius muscle-Sciatic nerve).
- (c) Frequency of tuning fork. ($n=100$ or $n=256$).
- (d) Your name, and the date on which the experiment was done.

Pass the paper, face upwards, through the fixing solution and let the excess drain off in to the tray. Dry it at room temperature, and cut out and trim the graph neatly before pasting it in the practical notebook.

This being the first experiment, the procedure has been described in detail. In the subsequent experiments only the relevant procedural steps will be described.

Observations and Results : You must observe your graph carefully. Is there anything unusual in the trace? Has the lever returned to the base line? The importance of meticulous and exact observations can hardly be overemphasised.

Application of a single adequate stimulus to the nerve causes a single, brief contraction of the muscle, followed by relaxation. The contraction does not begin immediately after the application of the stimulus but after a brief interval—this is the latent period. The portion of the curve labelled BE is the contraction phase, and ED is relaxation phase. BC and CD are contraction and relaxation periods respectively. Two small oscillations are seen after the relaxation is over. Do not draw vertical lines from points A, B, C and D onto the time trace for the calculation of various time periods. Instead, use a pair of dividers and count the number of waves for each period. The calculated durations in this experiment are : latent period=0.01 sec. (one wave) ; contraction period=0.04 sec. and relaxation period=0.05 sec. The total twitch duration is 0.1 second. Compare your results with the expected durations and if these vary greatly, try to find out the reason. NOTE : Sometimes the muscle starts giving spontaneous twitches. This is due to drying up of the nerve which is a physical stimulus. Injury to the nerve during dissection may also cause these.

Conclusions : The stimulation of the nerve generates nerve impulses which are transmitted to the muscle and the muscle contracts. Transmission of nerve impulses and electrical and chemical events in the muscle that precede mechanical activity are the cause of latent period. The oscillations seen after the twitch is over are due to jerking of the lever and have no physiological significance.

Discussion. The sciatic nerve is a mixed nerve, carrying motor or efferent fibres (axons of anterior grey column cells of the spinal cord) to the muscle fibres, and afferent fibres from the muscle spindles, tendons, ligaments etc. to the spinal cord. Stimulation of sciatic nerve sends nerve impulses down the efferent fibres to the neuromuscular junctions of muscle fibres where acetylcholine is liberated from the nerve terminals. If the depolarization at the motor end plate reaches—30 mv to—40 mv , it is propagated in both directions along the muscle fibres and the fibres contract. One skeletal muscle fibre has usually one motor end plate, rarely two. Thus, chemical and electrical events precede the mechanical events.

The latent period is due to : (a) Conduction of nerve impulses along the nerve fibres from the point of stimulation to the motor end plates, (b) electrical and chemical events in the muscle, (c) viscosity of the muscle, and (d) inertia of the lever system. If the muscle is stimulated directly (to exclude the time spent in conduction of nerve impulses), and if an optical recording system is employed (to exclude the inertia of the lever system), the latent period is very brief—this is the so-called 'true' latent period.

In the body, the muscle fibres are commonly activated by periods of repetitive stimuli in the form of trains of action potentials in the motor neurons. These are not necessarily in phase or at the same frequency from one neuron to another. The twitch is almost certainly not a physiological event. The usual repetitive stimuli come sufficiently close together so that the muscle does not relax between action potentials and each fibre produces a sustained contraction called tetanus. The twitch, however, is much easier to study outside the body as the muscle is less susceptible to fatigue.

Isotonic and isometric contraction. Muscle contraction in this experiment is of *isotonic* type as it lifts a weight and does external work, the tension remaining the same (isotonic). However, it is possible for the individual muscle fibres in a muscle to contract without any appreciable decrease in the length of the muscle as a whole.

For example, in attempting to lift a car the muscles generate great tension or force but cannot shorten. Contraction of muscles which maintain our posture against gravity is another example of contraction without shortening. This type of contraction is called *isometric* (same measure, length) contraction. The explanation of isometric contraction lies in the 'in-series' arrangement of the contractile elements (actin and myosin) with the elastic components of the muscle (tendon, connective tissue sheaths etc.). The increased tension of an isometrically contracting muscle (gastrocnemius of frog) can be recorded with an isometric lever, where both ends of the muscle are firmly fixed, and the tension is recorded through a mechanical lever or an electronic force transducer.

The sliding filament theory of muscle contraction. The link between depolarization of the sarcolemma and contraction (excitation-contraction coupling) is provided by the sarcoplasmic reticulum which is a highly specialised internal system of the muscle fibre. The spread of action potential from the sarcolemma down into the transverse tubules releases Ca^{2+} which binds to troponin. This is followed by a lateral shift of tropomyosin which results in the uncovering and binding of cross-linkages between the myosin and actin filaments. Splitting of ATP causes sliding of actin over the myosin filaments which brings about a shortening of the muscle fibre. Activation of Ca^{2+} pump returns the free Ca^{2+} from the sarcoplasm back into the tubular system and the muscle relaxes. ATP provides the energy for the mopping up of Ca^{2+} . Thus ATP is required both for contraction as well as relaxation.

Precautions. (1) Do not mishandle the nerve-muscle preparation. Keep it moist during the experiment. (2) Use extreme care while handling the kymograph which operates on 220 Volts AC. Ensure that it is properly earthed. (3) Do not remove the paper from the cylinder until it has been signed by a teacher, (4) Keep your working area on the table clean. Do not throw any discarded skin or muscle tissue pieces into the sink.

Questions : (1) Why is frog's muscle employed for muscle experiments? (2) Which stimulus is effective—'make' induced or 'break' induced, and why? (3) How are the muscles in the body activated? (4) What is the link between muscle excitation and contraction? (5) What is the total duration of the twitch and what are its time relations? (6) What is the cause of latent period? What is true latent period? (7) Is the contraction recorded by you isotonic or isometric? Give examples of such contractions in the body. (8) Is the contraction recorded by you an 'after-loaded' one or a 'free-loaded' one? (9) Name the contractile proteins of the muscle. What is the sliding-filament theory of muscle contraction?

Experiment No. : 1.5

**EFFECT OF CHANGING THE STRENGTH
OF STIMULUS ON MUSCLE CONTRACTION**

Principle. Single 'make' or 'break' stimuli of varying strength, starting with subthreshold, are applied to the nerve and the contractions of the muscle are recorded separately for each stimulus on a stationary cylinder.

Apparatus and reagents. As for simple muscle twitch.

Procedure. 1. Set up a nerve-muscle preparation and stimulation unit for recording single muscle contractions. *Exclude* the electrical contact terminals of the kymograph from the primary circuit so that induced current will be obtained only at 'make' or 'break' of the primary key.

2. Engage the gear lever at the neutral position and, with the writing point clear of the cylinder, move the secondary coil away from the primary to a point where no response occurs either at 'make' or 'break' of the primary key.

3. Draw a base line and start with subthreshold stimuli. Shift the secondary coil gradually towards the primary in steps of 3 cm and pass 'make' and 'break' stimuli at each position. Record the responses in pairs, by rotating the cylinder manually and note the distance of secondary coil at each position till it slips over the primary. With subthreshold stimuli, mark the point of stimulation *below* the base line otherwise it will be mistaken for a weak contraction (Fig. 1.8). This procedure excludes the necessity of including an event marker (electromagnetic signal marker) in the primary circuit.

4. Label the responses *M* and *B* and indicate the distance between the two coils.

Observations and Results : When the secondary coil is at 21 cm position, neither 'make' nor 'break' induced stimuli are effective i.e., these stimuli are *subminimal*. The first contraction, only a few millimeters in height, appears with a 'break' induced stimulus, with the secondary coil at 18 cm position ; the 'make'

stimulus is ineffective. This 'break' induced stimulus is the *threshold* stimulus. It can also be seen that this stimulus is stronger than the 'make' stimulus.

At this point you can also demonstrate the phenomenon of *summation of subminimal stimuli*. If you move the secondary coil to, say, 19 cm position where neither 'make' nor 'break' stimuli are effective (i.e., both are subminimal) and if you now rapidly pass 'make' and 'break' stimuli (by switching the simple key 'on' and 'off' a few times) these stimuli will summate and produce a contraction (this is not shown in the figure).

As the strength of stimulus is increased in steps the force of contraction goes on increasing, the 'break' contractions being stronger than the 'make' contractions. This occurs upto a certain limit beyond which there is no further increase in the amplitude of contractions.

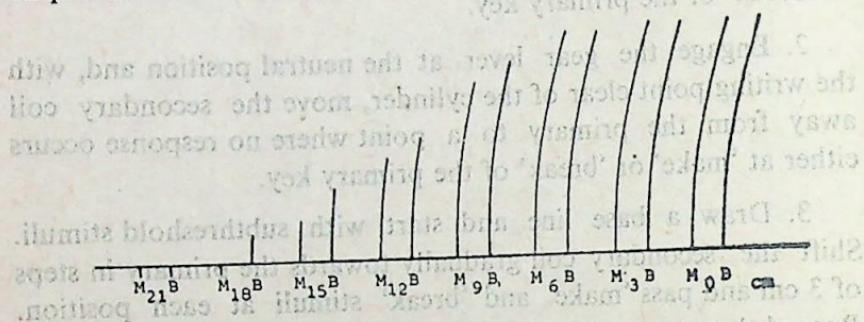


Fig. 1.8 : Influence of increasing strength of stimulus on skeletal muscle of frog. The first response appeared with a "break", induced stimulus, with the secondary coil 18 cm. distant from the primary.

Conclusions. It is obvious that 'break' induced stimulus is stronger than the 'make' induced stimulus. As the strength of stimulus is increased, more and more nerve fibres are excited and a larger number of muscle fibres contract. The last stimuli produce contractions of the same amplitude i.e., maximum responses. It can also be concluded that a maximal stimulus is the lowest strength of stimulus which produces a maximum response ; stimuli stronger than this do not produce any further increase in the size of the response and are called supramaximal stimuli.

Discussion The sciatic nerve contains motor fibres of varying excitability. Subthreshold stimuli fail to elicit propagated

impulses but with stronger stimuli, depolarizations in some nerve fibres (those having the lowest threshold) reach firing levels, transmitting impulses to many muscle fibres and the muscle as a whole gives a weak response. With further increase in the strength of stimulus, more and more nerve fibres are brought into action and the force of contraction goes on increasing till all the muscle fibres are contracting. This is sometimes called multifibre or quantal summation.

The 'all-or-none law' refers to the relationship between strength of stimulus and a 'unit-tissue, be it a nerve fibre, a skeletal muscle fibre, or the heart. Either the unit does not respond or else it responds to its best ability. The skeletal muscles as a whole does not obey this law, whereas a single muscle fibre does. This does not mean that the force of contraction of a fibre cannot be increased by any means, it only states that *this cannot be achieved by increasing the strength of stimulus*; for example, increase in the initial length of a muscle fibre does increase its force of contraction.

Gradation of muscular activity in the body. The force of muscular contractions in our body varies from very weak to powerful contractions. This gradation is achieved by (i) the number of motor units in operation, (ii) the frequency of nerve impulses and (iii) synchronization of impulses. It should be remembered that *there is no inhibitory nerve supply to skeletal muscle fibres*; relaxation is achieved by reduction of activity in the motor nerves and is a central phenomenon. In comparison, both cardiac and smooth muscle are supplied by inhibitory nerves.

Precautions. (1) The most important precaution is to bring the writing point in contact with the paper with the same force each time before recording the effect of a stimulus. Special stands are available for this purpose, though ordinary stands will serve the purpose if the student remembers this precaution. (2) The tilt of the cylinder should be so adjusted that the writing point remains in contact with the paper throughout its upward movement.

Questions : (1) Why does the force of contraction increase as the strength of stimulus is increased? What is multifibre or

quantal summation ? (2) Why does the force of contraction not increase after the strength of stimulus is increased beyond the maximal level ? (3) What is a motor unit ? What are the means by which the force of contraction of a muscle is graded in the body i.e., how is it that force of contraction of a muscle is weak sometimes and strong at other times ? (4) What is all-or-none law ? Does it apply to a skeletal muscle as a whole or to a single muscle fibre (unit tissue) ?

Experiment No. : 1.6

EFFECT OF TEMPERATURE ON MUSCLE CONTRACTION

Principle. A single contraction is recorded with the Ringer solution at room temperature. Keeping the point of stimulation unchanged, contractions are recorded at higher and lower temperatures. The effect of temperature on latent, contraction, and relaxation periods, and on the amplitude of contractions is noted.

Apparatus and reagents. As for simple twitch. Thermometer. Ringer solutions at room temperature (25°C — 30°C), 38°C and 5°C .

Procedure. (1) Record a simple muscle twitch, with the muscle immersed in Ringer at room temperature. Indicate the temperature on the tracing obtained. (2) Replace the solution with hot Ringer (38°C). Wait for about half a minute and record another contraction at this temperature without altering the point of stimulus. Note the temperature of Ringer with a thermometer. (3) Replace the Ringer with cold solution (5°C) and record a single contraction. (4) Draw lines from the summit of each tracing to the base line with the writing lever and

record a time-trace with a tuning fork for determining various periods (Fig. 1.9). Label your graph appropriately and fix it in the fixing solution. (5) Study the effect of Ringer at 50°C .

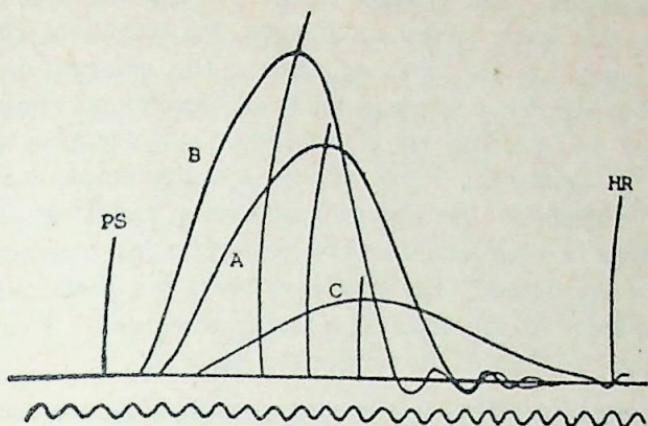


Fig. 1.9 : Muscle curves showing the effect of temperature on the amplitude of contraction and the time relations. PS—point of stimulation ; A—Ringer at room temperature (28° C) ; B—hot Ringer (38° C) ; C—cold Ringer (5°C) HR—muscle contraction as a result of heat rigor (50°C)

Observations and results. Compare the forms of the three muscle twitches which have the same point of stimulation. At higher temperature, the slope of the contraction phase is steeper, the latent period is reduced along with an increase in the amplitude of contraction. Contraction and relaxation periods are affected to a variable extent ; usually, these are decreased but may show an increase if the lever has been jerked up to a greater height and will, therefore, take a little longer time to return to the base line.

At low temperature, all the periods are prolonged, the amplitude is decreased and the contraction phase has a shallow slope. The lever may not return to the base line for an appreciable time at a temperature near 0°C .

When hot Ringer is poured on the muscle, the muscle proteins get coagulated and it shows irregular twitches and then shortens irreversible (it may elongate with time). This state is called *heat rigor*, a state from which there is no functional recovery.

Tabulate your results indicating the height in centimeters and duration in seconds for each contraction.

Discussion. The common effect of high temperature is to reduce the latent period and increase the amplitude while low temperature has the opposite effect. The decrease in latent period at high temperature is due to (a) decrease in viscosity of muscle, (b) hastening up of enzymatic and chemical changes prior to mechanical events and (c) speeding up of conduction of nerve impulses. The apparent increase in amplitude at 38°C is a lever artefact and does not represent a real increase in the force of contraction. The muscle contracts at a faster velocity, thus jerking up the lever to a higher amplitude. This is evident from the slope of the contraction phase.

Decrease in amplitude at low temperature is due to increased viscosity and slowing of chemical changes. If a contraction is recorded between 14°—18°C, it may show a better response than at 30°C.

The body temperature never rises to the extent at which heat rigor could occur. However, there is another type of rigor—called *rigor mortis*—that occurs after death. It occurs several hours after death when the muscles become rigid and shorten. The rigidity disappears after some time (depending on many factors) due to destruction of muscle proteins by enzymes that are released from cellular lysosomes. These changes in muscles i.e., appearance and disappearance of rigor mortis, help a forensic expert in fixing the time of death.

The effect of environmental temperature on muscular efficiency in an individual is entirely a different and complex problem. Physical efficiency depends on the state of nutrition, the type and severity of work, training and motivation, and environmental temperature and humidity. In very hot and humid weather the efficiency tends to decrease.

Precautions. (1) The point of stimulation for all the contractions should remain the same. (2) Maximal induction shocks should be used for each contraction to avoid beneficial effect. (3) The temperature of the Ringer solution should not exceed 42°C, otherwise heat rigor (heat coagulation of muscle proteins)

will result in irreversible shortening of the muscle. Heat rigor may, in fact, be demonstrated at the end of the experiment and the irreversible shortening of the muscle recorded on a stationary cylinder by pouring Ringer at 50°C on it.

Questions : (1) Why does warm Ringer increase the amplitude of contraction, and decrease the latent, contraction and relaxation periods ? Does the increased amplitude indicate a true increase in the force of contraction ? (2) How does warm saline affect the speed of contraction ? Can this effect be shown on your graph ? (3) How does low temperature affect the muscle twitch and why ? (4) What is heat rigor ? Is it a reversible process ? (5) What is rigor mortis ? What is its forensic importance ? (6) What factors affect muscular efficiency in man ?



Experiment No. : 1.7

VELOCITY OF NERVE IMPULSE

Two simple twitches are obtained separately by placing the electrodes on the nerve, first near the nerve-muscle junction and then near its vertebral end. The second contraction will show an increase in the latent period due to the time taken by the nerve impulse to traverse the distance between the two points stimulated. The velocity can be calculated by measuring the difference in the latent periods and the length of the nerve.

Principle. Two simple twitches are obtained separately by placing the electrodes on the nerve, first near the nerve-muscle junction and then near its vertebral end. The second contraction will show an increase in the latent period due to the time taken by the nerve impulse to traverse the distance between the two points stimulated. The velocity can be calculated by measuring the difference in the latent periods and the length of the nerve.

Apparatus. As for simple muscle twitch.

Procedure. Use a large frog so that its sciatic nerve has a substantial length. A frog muscle board is more convenient for this experiment. (1) Draw a base line and mark the point of stimulation. Place the stimulating electrodes on the sciatic nerve as near the knee joint as possible and record a simple

muscle twich. Keeping the point of stimulation unchanged, stimulate the sciatic nerve near the vertebral end and record another contraction (Fig. 1.10) (2) Run a time trace with a tuning fork, preferably of a high frequency ($n=256$). Measure

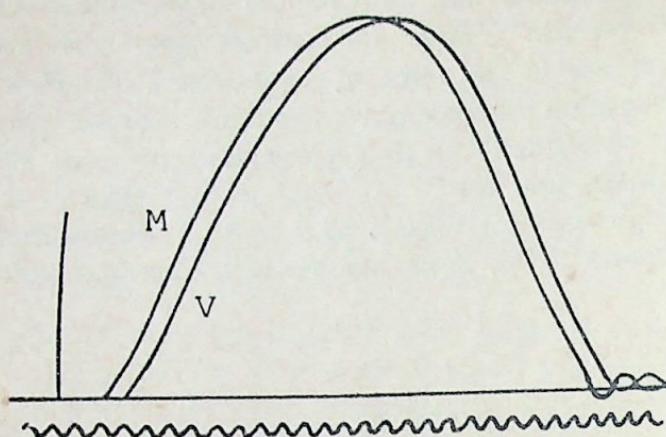


Fig 1.10 : Velocity of nerve impulse. Two single contractions successively recorded during stimulation of the nerve near the muscle end (M) and the vertebral end (V). Tuning fork—256 Hz.

the length of the nerve between the two points stimulated. Label the graph and enter the relevant data, especially the length of the nerve and the frequency of the tuning fork.

If possible, make two or three records and calculate the average of the velocities obtained.

Observations and results. Observe the difference in the two latent periods. Normally, the amplitudes of the two contractions are more or less the same. Calculate the durations of the two latent periods by using a pair of dividers. The difference is due to the time taken by the impulse to traverse the distance between the two points stimulated. For example, if $n=256$, length of the nerve = 6 cm, and there is a difference of one wave 1/256 sec to travel a distance of 6 cm. The nerve impulse has taken would be 256×6 i.e., 1536 cm/sec or 15.36 m/sec.

Conclusions. The experiment demonstrates that the conduction of the nerve impulse is not an instantaneous phenomenon. Considering that the electrical and chemical changes are identical in the two contractions, the difference in the two latent periods must be due to the transmission of the nerve impulses from the vertebral end of the nerve impulses to the other.

Discussion : Nerve fibres can be classified, according to their diameter, conduction velocities and thresholds of stimulation, into A, B and C groups. Group A fibres (diameter 12-20 μ m) are medullated and are further divided into α , β , γ and δ types. Group B (diameter 1-3 μ m) includes the preganglionic autonomic fibres, and group C fibres (diameter 0.5-2 μ m) are non-medullated. Nerve fibres may also be classified, according to their function into I, II, III, and IV types.

The frog's sciatic nerve is a mixed nerve, containing both sensory and motor fibres. In the present experiment, stimulation of the nerve causes nerve impulses (a nerve impulse is a propagated change in the membrane potential) to travel equally well in both directions from the point of stimulation (it is the stimulation of motor fibres that causes muscle contraction). However, in the intact animal, a nerve fibre conducts impulses in one direction only because it is always stimulated at the same end—the peripheral end in a sensory fibre and central end in a motor fibre.

The velocity of nerve impulses varies in different species, and in accordance with their diameter, thicker fibres conducting at a faster rate than thinner fibres (the velocity is approximately 5 meters per μ m diameter). In medullated fibres the nerve impulse jumps from one node of Ranvier to the next node—a process called *saltatory conduction*. The conduction velocity in different species is as under ; thick, medullated fibres (diam. 15—20 μ m) of mammals—100—120 m/sec. ; medullated fibres of frog—20—30 m/sec. ; non-medullated fibres (diam. about 1 μ m) of mammals—1-3 m/sec. The temperature also affects the conduction velocity—warming causing an increase and cooling a decrease in velocity.

Precautions. (1) The initial point of stimulus must not change. (2) The latent period of each contraction should be

measured separately and the difference calculated. (3) The cylinder should be firmly fixed to the spindle so that it does not rotate, even to a very slight extent, on the spindle.

Questions : (1) How are nerve fibres classified according to their diameter and function ? (2) What is a nerve impulse ? How is it conducted ? what is saltatory conduction ? (3) What are the factors that determine the velocity of conduction ? Which fibres conduct at the highest velocity in mammals ?

Experiment No. : 1.8

EFFECT OF TWO SUCCESSIVE STIMULI

Principle. Two successive stimuli are applied and the effect of the second stimulus arriving during the latent, contraction and relaxation phases of the contraction obtained with the first stimulus is recorded.

Apparatus. As for simple muscle twich. The striker or the dual electric contact arm fitted to the base of the spindle has two prongs, the lower being firmly screwed to the spindle while the upper prong can be moved back as desired. Two stimuli can thus be applied at varying intervals, the first stimulus (with the lower prong) arriving at the same point of stimulation each time.

Procedure. (1) Make the usual connections as before and use supramaximal stimuli. Draw a base line, mark the point of stimulus as *PS* and record a simple muscle twitch. (2) Move the upper prong back by about 1 cm so that the second stimulus would fall in the latent period of the previous tracing; mark the point of stimulation of the upper prong in the latent period. Record a contraction and label the point of the second stimulus and the tracing obtained *S₁*. The first stimulus arrived at *PS*

and the second at S_1 (3) Move the upper prong further back so that the second stimulus will arrive during the contraction phase. Mark and label the point of stimulation and the contraction obtained, S_2 . Record another contraction with the second stimulus arriving during the relaxation phase and mark the tracing and the point of stimulus, S_3 . (4) Finally, arrange

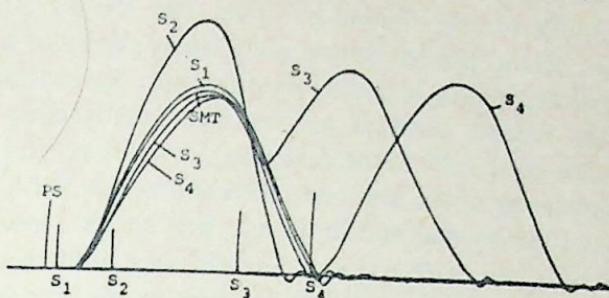


Fig 1.11 : The effect of two successive stimuli on muscle contraction. Five responses are shown. The first stimulus in each case falls at PS, the second stimulus arriving at S_1 , S_2 , S_3 , and S_4 . See text for details.

to pass the second stimulus after the first twitch is over and label these S_4 . (Fig. 1.11.) These contractions can also be recorded separately on different locations on the paper.

Observations and Results : It will be seen that when instead of a single stimulus, pairs of stimuli are applied to the sciatic nerve and these are so timed that the second stimulus falls during the different phases of the twitch that followed the first, the following effects are noted :

- (i) When the second stimulus falls during early latent period, it has no effect, the amplitude of contraction remains the same as that of the first twitch.
- (ii) When the second stimulus falls during the contraction phase of the first twitch, the muscle continues to contract and the trace shows an increase in amplitude (summation of contractions).
- (iii) When the second stimulus falls during the relaxation phase of the first twitch, the relaxation is arrested and the muscle contracts again, and with a greater force.

(iv) When the second stimulus arrives after the first twitch is over, there is another contraction, the force being more than that of the first.

Conclusions : When paired stimuli are applied to the sciatic nerve, the response of the muscle to the first stimulus affects its response to the second stimulus. That the second stimulus is ineffective when passed during early latent period of first twitch shows that the muscle is absolutely refractory during this period. When the second stimulus arrives during contraction phase of the first, *the contractile elements are further activated* and a second twitch grows out of the first one. This shows that the contractile elements of the skeletal muscle do not possess the property of refractoriness (the cardiac muscle is refractory to further stimulation throughout its contraction phase because the action potential lasts as long as contraction phase i.e., about 200-300 msec.). The bigger twitch in response to second stimulus falling after the first twitch is over is due to the beneficial effect of the first stimulus.

Discussion : The absolute refractory period of the skeletal muscle corresponds to the action potential the duration of which is about 1-3 msec. The mechanical response initiated by the first stimulus starts at this time and lasts for several milliseconds during which the effects of another stimulus can be added to it. The adding up of the effects of the two stimuli is called *summation* or *summation of effects*. The explanation for summation is as follows : the Ca^{2+} released into the sarcoplasm from the sarcoplasmic tubules during a contraction is mopped up by the same tubules during relaxation. When there is no relaxation or incomplete relaxation some Ca^{2+} remains behind in the sarcoplasm and this together with additional Ca^{2+} released with the second stimulus, makes more *activator* Ca^{2+} available to the contractile elements and the force generated is greater.

Decrease in viscosity and some rise in temperature resulting from the first stimulus may also contribute to the beneficial effect.

The next experiment studies the effect of many successively repeated stimuli on muscle contraction.

Questions : (1) What is the relation of electrical activity of skeletal muscle to its mechanical activity and how does it differ from that of the cardiac muscle ? (2) How does the response of a muscle to a stimulus affect its response to a second stimulus ? (3) What is meant by summation of effects ? What is the cause of beneficial effect ?

*Experiment No. : 1.9***GENESIS OF TETANUS**
(Effect of Many Successive Stimuli)

Principle. When the muscle is stimulated by a rapid succession of stimuli the muscle remains in a state of sustained contraction called tetanus. In this experiment, the frequency of stimulation is gradually increased from 8-10 to 40 or more per second.

Apparatus. As for simple muscle twitch. Variable interrupter or a vibrating reed. The purpose of these is to supply interrupted induced shocks at different rates.

Procedure. (1) Exclude the kymograph from the primary circuit but include a variable interrupter (the working of which has already been discussed) in its place. Adjust the kymograph speed to medium, i.e., 25 mm/sec. (2) Start with a low rate of stimulation by withdrawing the stop-screw away from the pendulum of the interrupter. Switch on the simple key and open the short-circuiting key. The pendulum starts vibrating slowly (8-10 times per sec.) and the muscle contracts with each induced shock. Record these individual twitches for about 2 inches on the paper. Swing the stylus off the cylinder. (3) Increase the rate of stimulation by pushing the stop-screw towards the pendulum, thereby decreasing the arc of its movements. Bring the writing point in contact with the rotating cylinder just ahead

of the previous tracing and record the contractions. Repeat this procedure twice or thrice, each time increasing the rate of stimulation, till the writing point inscribes a slowly rising smooth tracing on the paper. (5) Record a 0.5 second time-trace under the graph obtained. (Fig. 1.12.).

Observations and results. At low rates of stimulation (8-10/sec.), individual twitches are recorded while with increasing rates (15-20/sec.), the muscle does not relax fully before the next stimulus arrives, causing it to start contracting again. The resulting response is jerky and the tracing obtained shows a gradually increasing slope. With stimulation rates of about 40/sec or more, the successive stimuli arrive before the muscle begins to relax so that it remains in a state of sustained contraction. The plateau of the tracing exceeds the peaks of single twitches i.e., the muscle is contracting with a greater force. Stoppage of stimulation causes the muscle to relax immediately. (If stimulation were to continue, the plateau would be maintained until the muscle began to fatigue, at which time it would relax gradually).

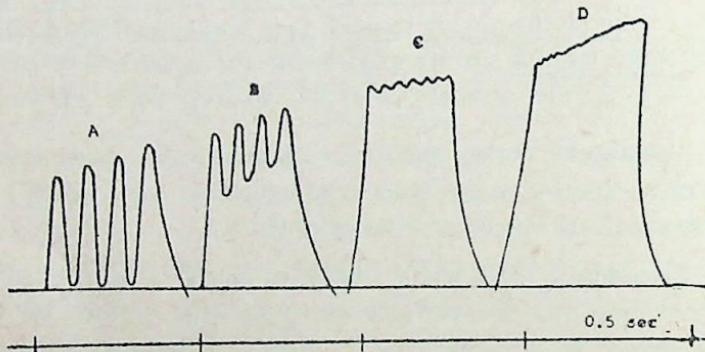


Fig 1.12 : Genesis of different forms of tetanus due to successive increase in the rate of stimulation. A—single contractions (note the beneficial effect); B—and C—subtetanus. D—complete tetanus. Time trace 0.5 sec.

Conclusion. As the twitch duration of gastrocnemius is 0.1 sec, any rates of stimulation less than 10/sec. cause individual twitches. Increased rates produce jerky or tremulous contractions called *subtetanus* or *incomplete tetanus*. The duration of contraction phase is 0.04 or 0.05 sec., thus stimulation rates of about 40-50 sec. or more produce complete tetanus.

Discussion. The contractile elements of the skeletal muscle do not possess the property of refractoriness. Repeated stimulation, therefore, results in adding up of the successive responses and the muscle exhibits one continuous contraction called tetanus. Thus, the rate of stimulation decides whether the responses are discrete, subtetanic or tetanic. Isometric recordings are ideal for demonstrating the considerable tension developed during tetanus as compared to individual twitches.

Just before the tetanizing rates, increased tension develops during each successive brief twitch, resulting in 'treppe' or 'staircase phenomenon'. This phenomenon can also be demonstrated in cardiac muscle (see chapter 2.9.) which, however, cannot be tetanized by repeated stimuli as it is completely refractory during the contraction phase.

In slow muscle fibres, tetanus may occur with frequencies of about 30/sec., whereas rapidly contracting fibres require 100 or more stimuli to produce tetanus. Furthermore, greater the frequency of stimulation greater is the tension produced which reaches a maximum that the muscle can develop. This is about 3 to 4 times the tension developed during a twitch.

Nature of muscle contractions in the body. Voluntary contractions of muscles in our body are not twitch-like responses and neither are they tremulous as have been recorded in this experiment. Weak muscle contractions result from a low-frequency (5 to 10/sec.) motor neuron discharge of a fraction of the motor neuron pool, but the expected jerky response is converted into a smooth contraction due to the asynchronous discharge of impulses from the motor neurons in operation. With increasing rates of impulse discharge the contractions become stronger until at and beyond the tetanizing frequencies (and, of course, with the recruitment of a large number of motor units) sustained and powerful contractions are produced. All muscle contractions in the body are tetanic in nature.

It may be recalled that the voluntary movements are voluntary in their aims and not in their means.

Questions : (1) What is subtetanus (sometimes called clonus) and tetanus ? How will you know from your trace that com-

plete tetanus has occurred ? (2) What is the staircase phenomenon (treppe) ? (3) Why does a tetanically contracting muscle generate greater tension than that of a simple muscle twitch ? (4) What is the nature of muscle contractions in the body i.e., are they twitches, subtetanic or tetanic ? (5) In the intact body, why is muscle contraction not jerky when the impulse frequency in the motor fibres is 10-20/second ?

Experiment No. : 1.10

THE PHENOMENON OF FATIGUE AND ITS SITE

Principle. If a muscle is stimulated repeatedly through its nerve, it undergoes fatigue i.e., it loses its physiological property of contraction.

Apparatus. The apparatus and the connections are the same as for simple muscle twitch. Use minimum amount of Ringer.

Procedure. (1) Draw a base line and mark the point of stimulation. Place the nerve on the electrodes and set the gear lever at the fastest speed. (2) Switch on the simple key and open the short-circuiting key. Every time the striker will complete the primary circuit, the muscle will contract. Start the kymograph and record a contraction. Stop the drum and label this contraction as 1. Switch on the kymograph again and record all the successive contractions until the muscle fails to respond. (3) Stop the kymograph and swing the lever off the cylinder. Engage the gear at 'neutral' and remove the nerve from the electrodes. Rotate the cylinder manually and bring the stylus in contact with the paper just ahead of the graph obtained. (4) Place the electrodes directly on the muscle and complete the primary circuit at the contact block by tapping

the spring with a finger. An induction shock reaches the muscle directly and its contraction is inscribed on the paper. Label this as 'direct stimulation' (Fig. 1.13.). (Repeated contractions of the muscle can also be recorded on a second kymograph with its cylinder moving at a slow speed. Another method is to record a contraction on the paper after every 20 contractions with the lever off the cylinder, till the muscle fails to contract).

The experiment has so far shown that muscle is not the seat of fatigue. That the nerve is not the seat of fatigue, can be demonstrated by bringing the sciatic nerve of a fresh prepara-

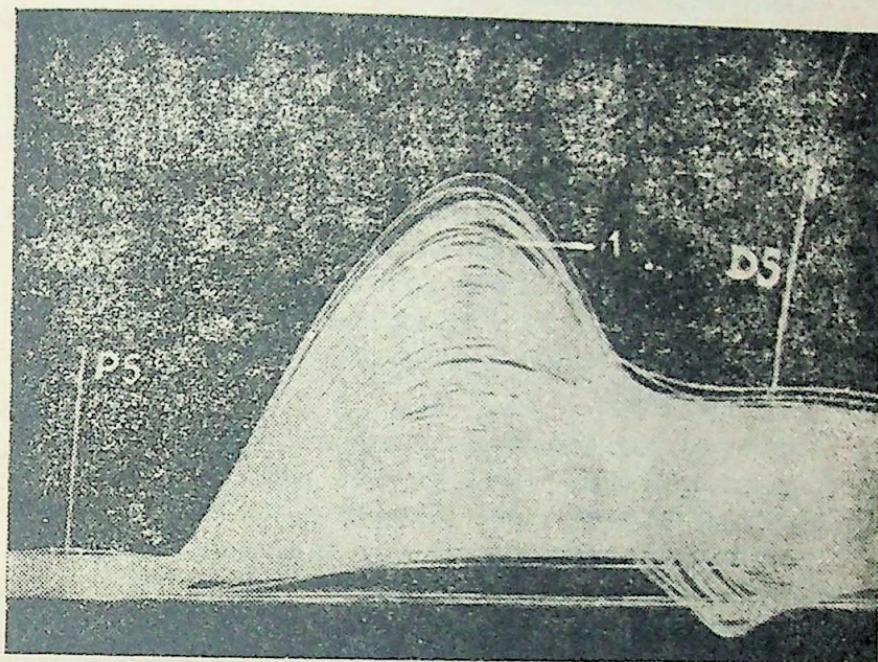


Fig. 1.13. : The phenomenon of fatigue. PS—point of stimulation. The record shows repeated contractions of the muscle through the stimulation of its nerve. The first contraction is labelled 1 ; the following few contractions increase in amplitude due to beneficial effect. As stimulation continues the muscle undergoes fatigue. DS—direct stimulation of muscle with the electrodes placed on it, after it had undergone fatigue through the stimulation of its nerve. This graph shows only that the muscle is not the seat of fatigue.

tion in contact with the nerve of the fatigued preparation, between the stimulating electrodes and the fatigued muscle. The second muscle responds every time after the completion of primary circuit, though the first muscle is not contracting. The first nerve in this case merely acts as an electrical conductor since it has no anatomical connections with the nerve and the muscle fibres of the fresh preparation.

Note the time for recovery from fatigue by stimulating the sciatic nerve at every two minutes interval.

Observations and Results : It is seen that the first few contractions increase in amplitude (the first contraction is marked 1). This is due to beneficial effect. This effect can be seen to better advantage if the contractions are recorded on a slowly moving drum. As the repeated stimulation continues, there is a progressive decrease in the amplitude and an increase in the latent, contraction and relaxation periods. Finally, the muscle fails to contract altogether and also the lever does not return to the base line. This inability of the muscle to relax fully is called *contracture*. (Recording every 20th contraction can give an exact idea of the effect of fatigue on various phases and time periods of the contractions).

After the muscle has undergone fatigue through stimulation of its nerve its direct stimulation with electrodes causes it to contract. If the muscle is allowed to rest for some time, contracture disappears and the muscle again responds to the stimulation of its nerve (it may be recalled here that while fatigue is a reversible process, heat rigor is not).

By employing a second nerve-muscle preparation, it is seen that the sciatic nerve is not the seat of fatigue.

Conclusions : It should be noted that the muscle in this experiment is a very artificial preparation. It has no circulation nor are its blood vessels being perfused. It is therefore quite a different 'type' of muscle as compared to those in the intact body.

Both the nerve and the muscle have been excluded as being the site of fatigue in this preparation. By the process of exclusion

the only possible site left is the neuromuscular junction. Obviously, some change has taken place here that is preventing neuromuscular transmission. Substances like lactic acid pyruvic acid and the breakdown products of ATP produced by repeated contractions may be interfering with the action of acetylcholine at the receptor sites (washing the muscle in saline can partially remove the waste products). There may be depletion of acetylcholine from the motor nerve endings, or the repeated depolarizations may be interfering with excitation, or the muscle receptors may become refractory to the transmitter. Thus, more than one factor may be causing neuromuscular block.

The phenomenon of neuromuscular fatigue can be studied in man by using Mosso's ergograph in which work is performed in lifting a weight, using a finger or thumb. The effect of median nerve stimulation and the effect of occlusion of brachial artery can also be studied.

Discussion. Direct stimulation of the muscle, after it has been fatigued through stimulation of its nerve, causes its contraction as this does not require the release of acetylcholine, the muscle fibres being depolarized directly.

If the direct stimulation of the muscle is repeated, the contractions become smaller, and, finally, the muscle fails to respond. When the stimulation is stopped, there is a gradual return of its excitability. Oxygen is essential for his recovery, because the fatigued muscle fails to recover if it is left in nitrogen. In this muscle preparation, fatigue may be considered to be due to the accumulation of metabolites in the muscle, possibly lactic acid. The oxidative removal of these substances allows the muscle to contract.

It is often mentioned that completely fatigued muscles after voluntary contractions can be made to contract by stimulation of their motor nerves and that fatigue is a result of some change in the central nervous system. These observations, and the conclusions drawn from them have been challenged. If for example, a muscular movement of the hand is carried on to complete fatigue, and the circulation in the arm is arrested at this moment with a blood pressure cuff, there is no recovery of strength until the blood flow is restored. It is obvious from this

that the site of fatigue must be in the muscles themselves and not in the central nervous system. Current evidence indicates that fatigue is a peripheral phenomenon occurring in the muscle itself.

Muscular fatigue experienced in severe exercise is due to events at the synapses in the central nervous system. This is a protective mechanism ; central fatigue is manifested before there is any block at the neuromuscular junction, and long before the muscle itself loses the ability to contract. Psychological factors like motivation and training as well as environmental factors like temperature and humidity affect muscular performance.

Questions. (1) What is meant by fatigue ? Is it a reversible process ? (2) What is the cause and seat of fatigue in this preparation ? How will you confirm that the nerve is not the seat of fatigue ? (3) What are the sources of energy for the contraction of muscle ? (4) What is the seat and cause of fatigue after heavy muscular work in man ?

Experiment No. : 1.11

EFFECT OF LOAD AND LENGTH ON MUSCLE CONTRACTION

Principle. A load can act on a muscle either before or after it starts contracting. In the former case, the load applied on the lever will stretch the muscle even before it starts contracting (free-loading), while in the latter case there is no initial stretching of the muscle fibres and load acts on the muscle after contraction has started (after-loading). Contractions are recorded under these two conditions using successively increasing loads. Work done by the muscle for each weight is then calculated.

Procedure. Set-up connections as before and use maximal stimuli, the intention being to record individual contractions with

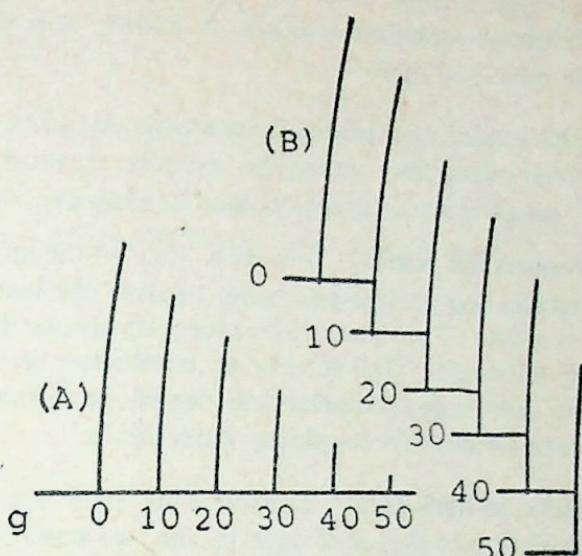


Fig. 1.14. : Effect of load and length on muscle contraction. (A)—after-loading ; (B)—free-loading. All contractions were recorded on a stationary drum, the free-loaded contractions being recorded from successively lower levels.

successively increasing loads, keeping the strength of stimulus, point of stimulation and the base line unchanged.

(a) After-loading. Ensure that the after-load screw is in firm contact with and supports the vertical arm of the writing lever. Apply a weight-hanger on the lever about an inch from the fulcrum, record a contraction and label this 0 g. Add a 10 g weight (the muscle is not being stretched or extended by the load), record a contraction and label this curve 10 g. Repeat this process with 20, 30, 40 and 50 g loads and label each trace accordingly.

(b) Free-loading (sometime also called pre-loading or fore-loading) Remove all the weights from the hanger, move back the after-load screw right up to the frame of the lever. The muscle is now being stretched by the load and the writing lever

moves down. Lift up the muscle trough to bring the writing point on the original base line and record a contraction. Record free-loaded contractions with 10, 20, 30, 40 and 50 g, raising the muscle trough each time a weight is added, thus stretching the muscle more and more.

The after-loaded and free-loaded contractions can be recorded on a stationary drum by rotating the cylinder forward by hand through 1 cm after each increase of load as shown in Fig. 1.14.

Observations and results. It is seen that in the after-loaded state the muscle does not have to work against the load until it begins to shorten. The lever rises more slowly as the load is successively increased. The velocity of contraction is seen to be more in the free-loaded contractions, the slope being steeper. This has been termed "force-velocity relationship."

Calculation of work done. The following data are required for the calculation of the work done in the two states of muscle contraction :

1. The height of contraction recorded for each weight (H).
2. Long arm of the lever (L) from the fulcrum to the writing point.

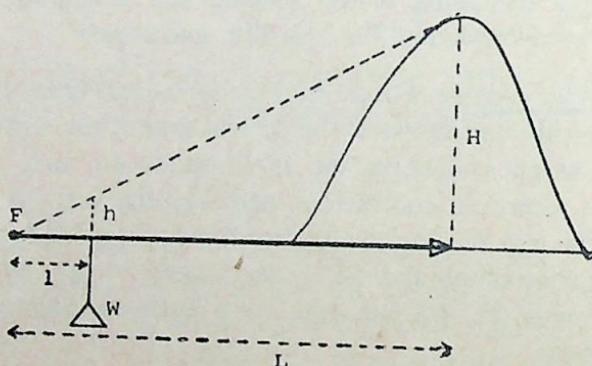


Fig. 1.15 : Calculation of the work done by the muscle. H —recorded height; h —the actual height through which the weight (W) has been lifted; L —long arm of the lever; l —short arm of the lever; F —fulcrum,

3. Short arm of the lever (l) from the fulcrum to the point where the load has been applied.
4. The actual height (h) through which the weight has been lifted (Fig. 1.15).

$$\text{The magnification of the lever} = \frac{L}{l}$$

Height of contraction = H on the record. Weight lifted = W . Work done = $Wg \times h$ cm

The actual height through which the weight has been lifted = h

$$\text{Work done} = W(g) \times h \text{ (cm)}; h = \frac{l}{L} \times H. \text{ Hence}$$

$$\text{the work done} = W \times \frac{l}{L} \times Hg \text{ cm.}$$

Multiply with 981 to express the result in ergs. Plot your result on a graph paper indicating the weights on the ordinate and the work performed on the abscissa.

Inference. The muscle does more work under "free-loaded" conditions than under "after-loaded". The muscular efficiency is greater in a "free-loaded" muscle only upto a certain physiological limit after which it starts decreasing. In the after-loaded muscle the efficiency may increase in the very early stages but it continues to decrease afterwards.

Discussion. When the muscle is stretched before contraction, its force of contraction is more. This is due to the greater initial length of the muscle fibres. The initial stretching of the muscle fibres reduces the overlap between the myosin and action filaments; this may possibly expose a greater number of binding sites between the contractile elements.

The lifting of a weight from the ground under normal conditions is an example of after-loading of the muscles. Under ordinary circumstances, the continuous pull of the antagonistic muscles in the body due to gravity exercises a stretching effect.

Thus our muscles are both "after-loaded" and "free-loaded" at the same time during many contractions.

Questions : (1) What is meant by free-loading and after-loading ? What will be the position of the after-load screw of the writing lever in either case ? (2) What is the effect of successive increase in load in either case ? (3) How is work done calculated ? (4) Do such contractions occur in the body ? Give examples of each. (5) What is optimum load ?

Experiments on Frog's Heart

Experiment No. : 2.1

EXPOSURE OF FROG'S HEART AND NAKED-EYE OBSERVATION OF ITS ACTIVITY

(a) Exposure of heart. Stun and pith a frog and lay it on its back on a dissection tray. Make a mid-line incision through the skin over the sternal region from the xiphisternum to the lower jaw. Extend the lower end of this incision laterally to the forelimbs and remove the triangular pieces of skin on both sides. The anterior chest wall is now exposed. Raise the xiphisternum with a forceps and give horizontal cuts through the muscle at this level. Cut through the pectoral girdle and anterior chest wall on each side with a bone forceps and remove the anterior chest wall in one piece. The heart is now revealed beating within the pericardial sac. (Do not cut through the anterior abdominal wall because the viscera will spill out.) Slit through the parietal pericardium and remove it right up to the base of the heart. Compare the size of the heart before and after the removal of the parietal pericardium.

(b) Naked-eye observation of the heart's activity. (1) The parietal layer of the membranous pericardium is easily removable but the visceral layer is firmly attached to the heart muscle. (2) The wall of the single ventricle is thicker than that of the atria. The truncus arteriosus lies anteriorly to the ventricle near its base

TA

2A 1TA
IV 1SV

and divides into two aortae. The colour of the ventricle is seen to become paler during contraction. Feel the ventricle between a finger and thumb and try to appreciate its hardening during each systole. You can see the sudden distension (diastole) of each cavity which quickly follows its contraction. (3) The two atria are separated from the ventricle by the atrio-ventricular groove. (4) The sinus venosus lies next to the atria and the superior and inferior vena cava empty into it. A careful observation will show a white crescentic line at the junction of the sinus and atria. (5) Sequence of the heart beat : The various parts of the heart contract in a definite sequence, starting with the mouths of the vena cavae followed by sinus, atria, ventricle and truncus arteriosus. The pauses between these contractions can be better appreciated if cold Ringer is poured on the heart to slow down its activity.

(c) Automatic contraction of the whole heart. Remove the heart from the animal's body and immerse it in Ringer solution placed in a petri dish. The various parts of the heart are seen to continue to beat in the usual regular sequence. This indicates that the cause of the heart beat lies in the heart itself.

The different parts of the heart. Observation (a) Cut through the sinu-atrial junction with a scissors separating the sinus from the rest of the heart. Count the rate of the sinus and compare it with that of the other portion of the heart. The rate of the sinus remains unchanged while the rest of the heart slows down, the atrial contraction preceding the ventricular beat.

Observation (b). Separate the atria from the ventricle by a cut placed just above the atrio-ventricular groove. The atria will be seen to beat at the previous rate but the ventricle stops after a few beats. The ventricle may resume beating after some minutes but at a still lower rate—the idioventricular rhythm.

Observation (c). Separate the apex of the ventricle with a fine scissors from the rest of the ventricle. It will be seen that the apical part usually does not resume contractions though it responds to a mechanical stimulus.

Conclusions and discussion. Three inferences can be derived from the observations made above ; these are :

1. Various parts of the heart contract in a regular sequence.
2. The heart will continue to beat outside the animal's body, in isolation from the nervous system. This indicates that the cause of the heart beat lies in the heart itself.
3. The property of self-excitation or automatic rhythmicity is maximum in the sinus, less in the atria and least in the ventricle. In the intact heart, atrial and ventricular activity is subordinated to the sinus and is controlled by it. This is called the sinus rhythm.

The cause of the heart beat is the spontaneous generation of rhythmic depolarizations, the pacemaker potentials, in the pacemaker tissue located in the wall of the sinus venosus. On reaching firing level, depolarization from this region spreads to the atria and then to the ventricle through muscle fibres connecting one chamber with the other. Each action potential is followed by a contraction response. There is no special conducting tissue comparable to the bundle of His found in the mammalian heart.

There is no coronary circulation in the frog's heart; the hierarchy of pacemaking in the cardiac chambers can, therefore, be easily shown in a simple experiment (see experiment 2.9).

Experiment No. : 2.2

NORMAL CARDIOGRAM

Cardiogram is a record of the mechanical activity of the heart. A Starling's heart lever is attached to the apex of the ventricle and the movements of the lever are recorded.

Apparatus. Kymograph. Starling's heart lever. Frog board or muscle trough. Electromagnetic time signal maker. Ringer solution. Speed of the kymograph—1.2 mm.

Procedure. 1. Expose the heart of a frog and transfer the animal to a frog board or muscle trough. Fix the heart lever on the stand above the frog and pass the hook of the pin through the apex of the ventricle. Lift the heart gently by raising the heart lever and adjust its position so that the beating heart produces good movements of the writing lever. Contraction of the heart pulls the lever downwards whereas during diastole the spring of the heart lever assembly pulls the lever back to its mean horizontal position. Pour Ringer solution on the heart from time to time with a dropper or cotton wool.

2. Bring the writing point of the lever in gentle contact with the recording surface at a tangent, with minimum of friction. Note the sequence of contractions of the cardiac chambers and record these events as shown in Fig. 2.1. Only a preparation handled carefully will show the various waves. It should be remembered that the tracing obtained is the algebraic sum of the movements of the various parts of the heart, so that, if one chamber is contracting while another is relaxing, the writing lever may move very little.

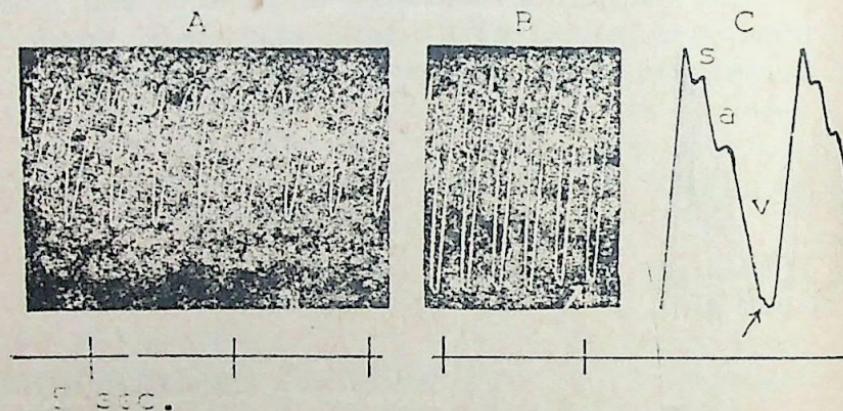


Fig. 2.1 : Record of spontaneously beating heart of frog.

- (A) In this trace only the atrial and ventricular events are seen.
- (B) In this trace, recorded from another frog, contractions of sinus (S), atria (a) and ventricle (V), followed by relaxations, can be seen as shown in the diagram (C). Contraction of truncus arteriosus occurs just before the beginning of ventricular diastole (arrow).

3. Connect a signal marker to the time signal terminals on your seat and record a time trace of 5 seconds below the cardiogram. Label the various components of the cardiogram, enter the relevant data on your graph and get your experiment approved and signed by a teacher. Remove the paper and fix it in the usual way.

Observations and Results : Note if the contractions of all the chambers have been recorded in your experiment. Look carefully at the entire trace and see whether the rhythm is regular by counting the heart rate at two or three places on the trace. Also note if there is any difference in the trace before and after pouring saline on the heart.

The heart rate in summer is usually between 40 and 50 minute. In winter it slows down.

Discussion. The cardiac impulse (wave of depolarization) passes quickly over the atria and on to the ventricle from muscle cell to muscle cell. The slight pause between the atrial and ventricular contractions is due to a normal partial block to the passage of the excitatory wave. There is no definite fibrous ring between the atria and the ventricle, though there is muscular continuity. The muscle fibres at this junction run circularly around the heart and not directly from the atria to the ventricle. This may account for the delay between the atrial and ventricular contractions. In a normal cardiac cycle the sinus possesses the highest frequency of rhythm than the other chambers and therefore determines the rate of the heart—thus acting as the pacemaker.

Questions : (1) Will the heart continue to beat if it is taken out of the animal's body and placed in a suitable solution ? What is the cause of heart beat ? What is the role of the nerve supply to the heart ? (2) Name the different chambers of the frog's heart and compare these with the mammalian heart. (3) Where is the pacemaker of the frog's heart situated ? How does the cardiac impulse (the wave of depolarization) spread from the pacemaker to the different chambers ? Is there a special conducting system like the one in the mam-

malian heart ? (4) What is the difference between cardiogram and an electrocardiogram ? (5) Enumerate the different properties of cardiac muscle ? How does this muscle differ from the other types of muscle tissue ?

Experiment No. : 2.3

THE EFFECT OF TEMPERATURE ON FROG'S HEART

Apparatus : As for normal cardiogram. Hot and cold Ringer.

Procedure (1) Set up your apparatus for recording the normal cardiogram. Pour Ringer at room temperature (say at around 30°C (in winter) and record a few contractions. (2) Stop the kymograph. Pour Ringer at about 38° C on the heart drop by drop. The rate and force will be seen to increase (Fig. 2.2.) When the effect is maximum, switch on the kymograph and record a few beats. (3) Stop the kymograph once again, and pour Ringer at room temperature on the heart till it resumes the previous rate and force. Now pour Ringer at about 5°C on the heart drop by drop. The rate and force will be seen to decrease.

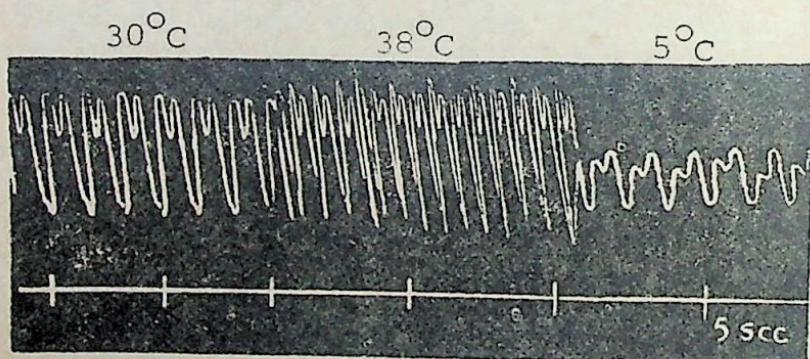


Fig. 2.2 : Influence of change of temperature on the frog's heart. Note the change in rate and force of contraction with hot and cold Ringer.

When the effect is maximum, switch on the kymograph and record a few beats. (Alternatively, the effects of hot and cold Ringer may be studied on a continuously moving paper). (4) Record a time trace below the graph. Enter the relevant data, put arrows where hot and cold Ringer was added, remove and fix the graph in the usual manner. Tabulate your results showing the effect of temperature on rate and force of the heart best.

Discussion : At high temperatures the chemical reactions and the metabolic activity of the pacemaker tissue are accelerated. This results in the generation of more cardiac impulses per unit time thus increasing the heart rate. Increased metabolic activity of the cardiac muscle itself causes an increase in the force of contraction. Low temperature has the opposite effect.

Questions : (1) What is the site of action of temperature on the beating heart ? (2) How can you show, by looking at the graph, that the rate and force have decreased or increased ?

Experiment No. : 2.4

THE EFFECT OF ADRENALIN AND ACETYLCHOLINE OF THE HEART

Procedure : (1) Record some normal beats. Stop the kymograph and pour freshly prepared solution of 1 in 10,000 adrenalin on the heart drop by drop and note the effect. Both the rate and force will show an increase (Fig. 2.3.) (2) Stop the kymograph and wash away the drug with Ringer. Pour a few drops of 1 in 100,000 acetylcholine (ACh) solution on the

Ad \rightarrow Rate (+), Force (+)
ACh \rightarrow Rate (-), Force (-)

heat and note its effect. This drug inhibits the heart and both the rate and force decrease. As the heart is beating, pour 0.5 per cent atropine sulphate solution on it—there will be no effect i.e., the inhibition will continue. (3) Stop the kymograph. Wash the heart with Ringer till normal beats are restored. Pour atropine solution on the heart and note that the heart continues to beat normally. Now pour acetylcholine on the heart and watch for its effect—the heart is not inhibited now. Put arrows on your graph to indicate the places where a particular drug was poured. (4) Record a time trace below your graph and remove and fix the paper as usual. (5) Tabulate your results indicating the effects of drugs on the rate and force of heart.

Conclusions : Adrenalin, a sympathomimetic drug, increases heart rate by its action on the pacemaker and the force of contraction by its direct effect on the cardiac muscle. Acetylcholine (ACh), a parasympathomimetic drug, inhibits the heart, decreasing its rate by its action on the pacemaker and force by its direct effect on the muscle.

When atropine, a parasympatholytic drug, is poured on the heart after ACh it does not block the inhibitory action of ACh.

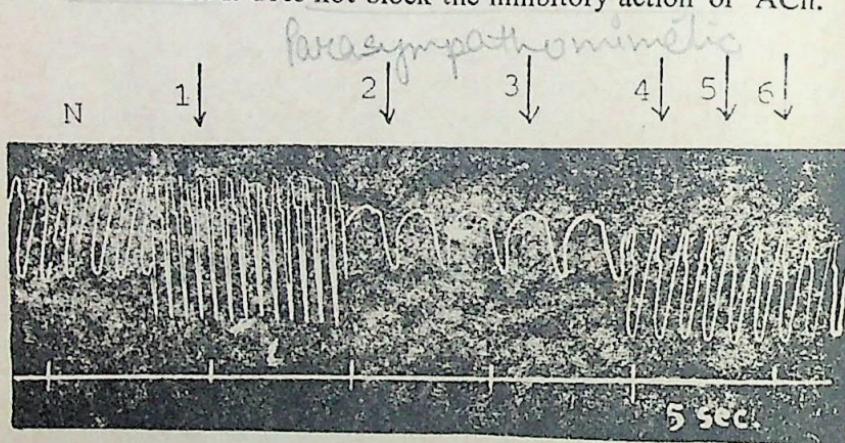


Fig. 2.3 : Effect of adrenalin and acetylcholine on the frog's heart N-normal cardiogram : Arrows—1-adrenalin; 2-acetylcholine ; 3-atropine poured on the beating heart ; 4-normal cardiogram ; 5-atropine ; 6-acetylcholine. See text for details.

However, when atropine is applied before ACh, it blocks the inhibitory effect of ACh, and as a result the heart continues to beat normally. Atropine blocks the action of ACh by attaching itself to the membrane receptors of the cardiac muscle fibres.

Discussion : Acetylcholine has two types of actions—*muscarinic* and *nicotinic*. Muscarinic actions (muscarine is an alkaloid obtained from a poisonous mushroom) are those that it exerts on cardiac muscle, smooth muscle, and exocrine glands. These actions are slow to start and last for a long time. These actions of ACh are antagonized by atropine. Nicotinic actions of ACh (nicotine is an alkaloid of tobacco) are those that it exerts on postganglionic neurons of autonomic ganglia (these actions are antagonized by hexamethonium group and other drugs), and on the motor and plates (these actions are antagonized by curare and other neuromuscular blocking drugs).

In the body, acetylcholine is released at many sites—e.g., motor end plates, endings of both preganglionic sympathetic and parasympathetic neurons, endings of postganglionic parasympathetic neurons, endings of some of the postganglionic sympathetic neurons (those supplying blood vessels of skeletal muscles, sweat, glands, pilomotor muscles), and at some synapses in the central nervous system.

Adrenalin (methylnoradrenalin) is one of the catecholamines. It is the main hormone of adrenal medulla, being formed from phenylalanine. It is also one of the synaptic transmitters.

Questions : (1) What are the main actions of ACh ? How can these actions be blocked ? (2) What are the sites in the body where ACh is released ? (3) What are catecholamines and what are their actions in the body ? (4) How do these drugs affect the rate and force of heart beat in the frog ?

↓ ↓ ↓ ↓ ✓

Rate — S.V
Force — muscles

Experiment No. : 2.5

REFRACTORINESS OF CARDIAC MUSCLE EXTRASYSTOLE AND COMPENSATORY PAUSE

Procedure : 1. Adjust the strength of stimulus so that both 'make' and 'break' induced shocks are strong enough to cause the contraction of the ventricle when applied to it during diastole. Include a signal marker in the primary circuit to indicate the exact moment in the cardiac cycle at which a stimulus will fall.

2. Transfer the frog to a muscle chamber and attach the heart lever to the ventricle. Adjust the signal marker so that it writes about half an inch above and in the same vertical line as the tip of the writing point.

3. Bring the stimulating electrodes in gentle contact with the ventricle and watch the excursions of the heart lever carefully. Send in an induction shock during systole, and then, a few beats later, in early diastole, and then in mid and late diastole. Note a compensatory pause after each extrasystole (Fig. 2.4.)

Observations and results : It is seen that when the shock falls during any part of systole, it is ineffective and the heart continues to beat as before. When the stimulus falls during very early diastole, there is no response in most cases unless the stimulus is very strong. If the stimulus falls during mid or late diastole, the heart responds by contracting. This contraction is called an extrasystole (extra contraction). It will be seen that an extrasystole is followed by a compensatory pause after which the heart resumes normal beating.

Discussion : The contractile response of the heart begins soon after the onset of depolarization and continues into the plateau of repolarization which is a triphasic and slow process. Because

of this the heart does not respond to any stimulus during systole and diastole has to start before the heart muscle can respond to any stimulus. Obviously, the heart muscle has a long refractory period and so cannot be tetanized. Were it possible to tetanize the heart, it would have lethal consequences in that it would lose its only function—that of muscular pump, which would bring the circulation to a standstill.

The compensatory pause following a premature beat is due to the fact that when the cardiac impulse from the sinus reaches the ventricle, it finds it in a state of absolute refractoriness (during the extrasystole); the ventricle has to wait for the next spontaneous impulse to arrive from the pacemaker before it can respond by automatic contraction. The first contraction after the pause is often more forceful than a normal beat. Possibly, the electrical potential of the premature beat releases some substances favouring the energy of contraction.

Extrasystoles are commonly encountered in medical practice, the common causes being myocardial infarction, digitalis therapy, excessive smoking etc. The extrasystoles may occur in otherwise normal people. Some ectopic focus in the heart generates an impuls which produces a premature contraction which is followed by a compensatory pause. Electrocardiography reveals the atrial or ventricular origin of the extrasystoles.

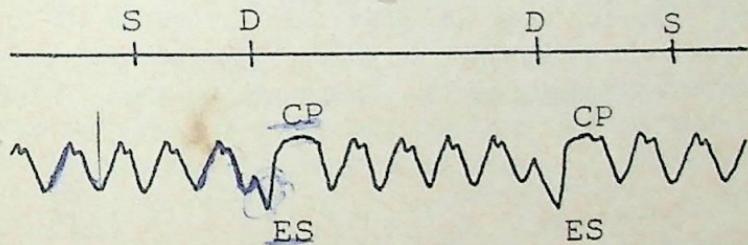


FIG. 2.4. : Refractoriness of the heart muscle. The signal trace indicates the moment of stimulation of the ventricle during the cardiac cycle. Outside stimulation during systole (S) has no effect ; stimulation during diastole (D) produces an extrasystole (ES) which is followed by a compensatory pause (CP)

Questions : (1) Why is an external (outside) stimulus ineffective when applied to the beating heart during systole ? (2) What is the practical value of the property of refractoriness of the heart muscle ? (3) What is the cause of compensatory pause that follows an extrasystole ? (4) What is the relationship between electrical and mechanical events of the heart ? Draw the diagram of a pacemaker potential. (5) What are the causes of extrasystoles in man ? How are the different types identified ?

Muscarine
Experiment No. : 2.6

THE EFFECT OF VAGOSYMPATHETIC AND WHITE CRESCENTIC LINE STIMULATION

Innervation of the heart : The frog's heart is supplied by both sympathetic and parasympathetic divisions of the autonomic nervous system. The origin of sympathetic (accelerator) and parasympathetic vagal (depressor) fibres is in the central nervous system. The two types of fibres are intermingled in the vatosympathetic trunk, which, therefore, contains post-ganglionic sympathetic and preganglionic parasympathetic fibres. Vagal fibres enter the white crescentic line and make synaptic connections with the postganglionic neurons which supply the heart.

Exposure of vatosympathetic trunk. (1) Expose the heart as before and proceed to clean and expose the petrohyoid muscle. This is a narrow strip of muscle running from the base of the skull towards the hyoid bone and crosses a very shiny tendon of a deep-lying muscle. (2) Lift up the lower border of petrohyoid with a forceps ; the vatosympathetic trunk, carotid artery and the laryngeal branch of the vagus will be revealed crossing

the shiny tendon at right angles. Do not try to clean and separate these structures. Expose these on the other side as well. Push a blunt glass probe under these and tie loose knots around these structures with pieces of thread for easy location and stimulation later. Confirm the identity of the exposed vagus by stimulating it and watching the heart stop before transferring the animal to a muscle trough.

Procedure. Include a signal marker in the primary circuit in addition to a variable interruptor.

(A) *Stimulation of vagosympathetic trunk.* (1) Adjust the signal marker and the heart lever so that their writing points are in a vertical line. (2) Apply the stimulating electrodes to the vagosympathetic trunk taking care that they do not touch the surrounding muscles. (3) Record a few normal beats, switch on the stimulation and record the stoppage of the heart for a few seconds. Turn off the stimulation ; the heart begins to beat again, usually more vigorously than before. In the event that the heart does not stop, stimulate the vagus on the opposite side. Weaker stimulation may only slow the heart.

(B) *Stimulation of the white crescentic line.* Apply the tips of the electrodes on the white crescentic line, without disturbing the heart ; turn on the stimulation and record the effects (Fig. 2.5).

Discussion. Vagal fibres are inhibitory to the heart, and though these are mixed with the sympathetic fibres, the common

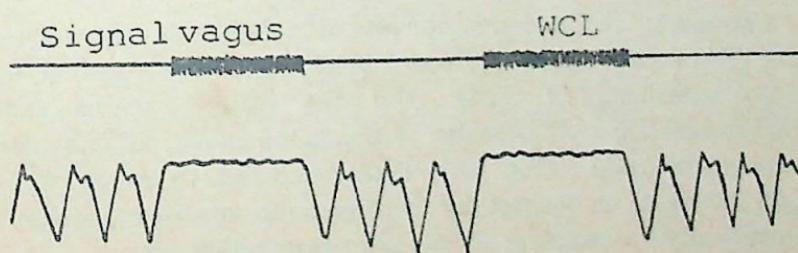


Fig. 2.5. : Inhibition of frog's heart during vagal and white crescentic line (WCL) stimulation. The heart is arrested in diastole in both cases. The signal trace indicates the duration of stimulation in both cases. Note that after stoppage of vagal stimulation, the force of cardiac contraction increases.

effect of stimulation of the vago-sympathetic trunk is stoppage of heart. The vagal fibres are not, however, equally distributed between the two trunks, which explains why no inhibition of the heart results from stimulation on one side while the opposite trunk is strongly inhibitory. Sympathetic effects are frequently revealed after the stoppage of stimulation when the heart resumes its beat with more vigor.

Stimulation of the white crescentic line excites the post-ganglionic neurons directly. Acetylcholine is the transmitter at the endings of both pre and post-ganglionic vagal fibres whereas noradrenalin is the transmitter at the endings of post-ganglionic sympathetic fibres.

Experiment No : 2.7

THE PHENOMENON OF VAGAL ESCAPE

Strong stimulation of the vagus stops the heart but if the stimulation is continued over a period the heart escapes from its inhibitory effect and starts beating once again.

Procedure. Set up connections for stimulation of the vagus as in previous experiment. Record some normal beats and turn on the stimulation of vagus. The heart stops. *Continue* the vagal stimulation even after the stoppage of heart till it starts beating once again (Fig. 2.6). If the heart fails to escape from vagal inhibition on one side try the stimulation on the other side. Occasionally the escape is not obtained from either side.

Discussion. Many factors may contribute to the escaping of the heart from the inhibitory effect of vagal stimulation. The cardiac muscle may become refractory to the action of acetylcholine, or there may be exhaustion of the transmitter in the vagal

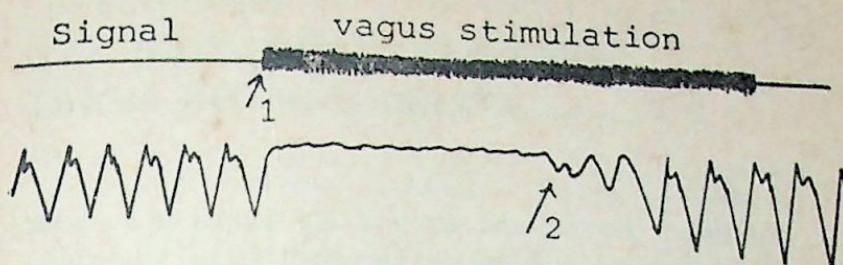


Fig. 2.6. : Stoppage of heart in response to vagal stimulation and its escape from the inhibitory effect of vagus. The arrow 1 indicates the beginning of vagal stimulation and stoppage of heart in diastole. The heart escapes from this inhibition though the vagal stimulation is continued—the escape is shown at arrow 2.

fibers so that idioventricular rhythm starts, the heart beating at a very slow rate. The sympathetic effect may overpower the vagal effect thus releasing the heart from inhibition. The tracing obtained by the student will usually indicate the possible cause of escape in most cases.

Questions : (1) How would you identify the vago-sympathetic trunk ? Which types of fibres does it carry to the heart and what is their role in the activity of the heart ? (2) Does the vagus supply the ventricle (or ventricles in mammalian heart) ? If not, why does its stimulation stop the ventricular activity ? (3) What is the significance of white crescentic line ? Name the parasympathetic ganglia of the frog's heart. (4) When vagus (or white crescentic line) is stimulated does the heart stop in systole or diastole and why ? (5) What is vagal tone ? What is its cause ? Is there any vagal tone in the frog under study ? (6) What is meant by vagal escape (what escapes what) ? What are the causes of vagal escape.

Experiment No. : 2.8

THE EFFECT OF NICOTINE AND ATROPINE ON THE HEART

Principle. Nicotine and atropine are poured on the heart and their site of action is revealed by stimulation of the vagosympathetic trunk and the white crescentic line.

Apparatus. Recording and stimulating units. 1 per cent solution of nicotine and 0.5 per cent solution of atropine sulphate.

Procedure. Note that the stimulation of vagosympathetic trunk represents stimulation of preganglionic fibres while stimulation of white crescentic line represents stimulation of postganglionic vagus neurons. (1) Set up the experiment as for the stimulation of vagus and white crescentic line. Record a few normal beats and stimulate the vagus and the white crescentic line to confirm their inhibitory effects. (2) Pour a few drops of nicotine solution on the heart, stimulate the vagus after a few seconds, and note that the heart does not stop. After a few more beats, stimulate the white crescentic line and note the stoppage of heart in this case. (3) Wash away the drug with Ringer and pour a few drops of atropine solution on the heart. Wait for a few seconds and then stimulate the vagus, the heart does not stop. Stimulate the white crescentic line a few beats later and note that the heart is not inhibited. Consult Fig. 2.7 and compare your results with those shown here.

Discussion. It will be noted that the nicotine and atropine solutions do not alter the activity of the heart which continues to beat as before. (There is no vagal tone in this preparation since the central nervous system has been destroyed).

1. When the heart has been treated with nicotine it is seen that :

- (a) Stimulation of vagus does not inhibit the heart, and
- (b) Stimulation of white crescentic line inhibits the heart.

It is obvious that nicotine has blocked the transmission of impulses from preganglionic to postganglionic neurons at the white crescentic line, whose stimulation, which excites the post-ganglionic neurons directly, still inhibits the heart.

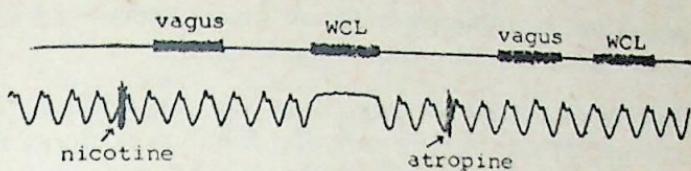


Fig. 2.7. : Frog's heart treated with nicotine and atropine followed by stimulation of vagus and white crescentic line (WCL) in each case. The experiment is designed to show that the vagus nerve is interrupted on its way to the heart. See text for details.

2. Treatment of the heart with atropine reveals that :

- (a) Stimulation of the vagus does not inhibit the heart, and
- (b) Stimulation of the white crescentic line also fails to stop the heart.

These observations show that block of inhibitory impulses is beyond the white crescentic line i.e., at the endings of the post-ganglionic fibres. This experiment shows, therefore, that the vagus nerve is interrupted in its course to the heart, and that the site of interruption is at the white crescentic line.

Questions : (1) What type of drug is atropine ? Where and how does it act to block the action of acetylcholine ? (2) Where does nicotine act to block the action of acetylcholine ? (3) What conclusion would you derive from this experiment ?

Experiment No. : 2.9

STANNIUS LIGATURES

All or None Law Summation of Stimuli and Staircase Phenomenon

Principle. The normal spread of cardiac impulse from the sinus to atria to ventricle can be blocked by tying ligatures at

the junction of these chambers. The different rates of impulse generation from these chamber as well as some properties of heart can be demonstrated.

Apparatus. Arrangement for obtaining stimuli of varying strength. Stout cotton thread. Signal marker.

Procedure. Expose the frog's heart and pass a ligature (8" to 10" of stout thread) with the help of a fine forceps, dorsal to the truncus arteriosus and ventral to the atria; leave the thread in position.

Ist Stannius ligature. Transfer the animal to the muscle chamber, and attach the heart lever to the ventricle without stretching it and record a few contractions. With the drum running, and while the heart continues to beat, bring the two ends of the thread forward and tie a tight ligature with a single knot over the white crescentic line. If the ligature has been applied properly, it will be seen that the atria and ventricle stop in diastole after one or two beats but the sinus continues to beat as before. Such a heart preparation is called a "Stannius heart". After a variable interval of 5 to 20 minutes the heart starts beating again, the atrial contraction followed by the ventricular beat. Record these contractions. It will be seen that the sinus is beating at a faster rate than the rest of the heart (Fig. 2.8).

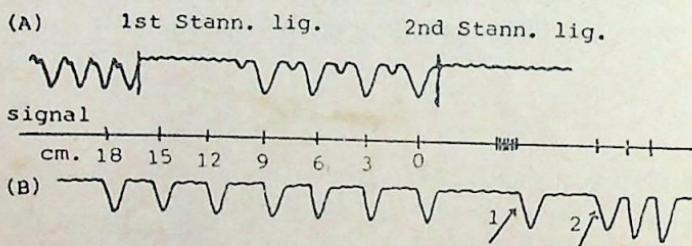


Fig. 2.8 : Stannius ligatures, showing hierarchy of pacemaking in the heart. (A)—sinus and rest of the heart beating at different rhythms after 1st Stannius ligature. The ventricle stops beating after 2nd Stannius ligature (B)—The inactive ventricle is employed for demonstrating all or none law i.e., with increasing strength of stimulus the force of contraction remains unchanged; nomenon (arrow 1); and staircase phe-

In a modification of the above experiment, a state of partial heart block can be created and recorded in a separate experiment. Tie only a loose knot to begin with and see if there is any slowing of the atria and ventricle, though the sinus will continue to beat at its previous rate. Study the effect of gradually tightening the ligature, in steps, until the atria and ventricle stop altogether. Make similar observations with the second Stannius ligature (see below where to tie it).

2nd Stannius ligature. Tie a tight ligature at the atrioventricular junction. The ventricle stops beating immediately or beats a couple of times and then stops; the sinus and atria continue at their own inherent but different rhythms.

While the ventricle is quiescent following the 2nd Stannius ligature, carry out the following experiments :

1. **Summation of subminimal stimuli.** Include the signal marker in the primary circuit and apply the stimulating electrodes on the ventricle. Move the secondary coil away from the primary and select a stimulus that just fails to evoke a response. Bring the writing lever in contact with the drum and 'make' and 'break' the simple key rapidly so that many subminimal stimuli are passed at very short intervals (say half a second). It will be seen that after a variable number of stimuli (5 to 10) the ventricle responds by contraction. If there is no response increase the strength slightly but even then neither 'make' nor 'break' stimulus should produce a response individually. Repeat the stimulation of the ventricle as before.

2. **All or none law.** Start with a subthreshold stimulus and mark it as 'M' or 'B' as the case may be and record the position of the secondary coil from the scale. Continue to move the secondary coil towards the primary in steps of 3 cm and go on recording the responses of the ventricle to these single induction shocks. The signal marker will indicate, each time, the point where a stimulus was passed. In this way the ventricle will be stimulated by *successively stronger stimuli*. It is essential to allow at least 10 seconds interval between each stimulus to avoid beneficial effect.

3. Staircase phenomenon. Adjust the position of the induction coil to get single shocks both at 'make' and 'break'. Apply 3 or 4 stimuli, one after the other, so that a fresh contraction occurs immediately after the previous contraction and relaxation is over. There is a gradual increase in the force of contraction of the ventricle (Fig. 2.8). This does not go against the all or none law but is due to beneficial effect.

Discussion. In contrast to skeletal muscle, the cardiac muscle is not organised on a muscle unit basis and behaves as a physiological syncytium. As long as the stimulus is below threshold, there is no contraction. With a threshold stimulus, as also with stimuli of increasing strength, all myocardial cells are activated during each contraction in an all or none fashion i.e. there is no increase in the force of contraction with increasing strength of stimuli. Thus the heart either does not respond to a stimulus but if it does it responds to its best ability. The all or none law does not mean that the force of cardiac contraction cannot be increased by any means, it only means that it cannot be achieved by increasing the strength of stimulus.

Questions : (1) What is the purpose of applying Stannius ligatures ? Can this experiment be done on a mammalian heart ? If not, why not ? (2) Why do the atria and ventricle stop beating after first Stannius ligature ? Why do these chambers start beating after a while ? (3) Why does the ventricle stop beating after the second Stannius ligature ? What is its rate after it resumes its activity ? (4) Why is sinus venosus (and the SA node in the mammalian heart) normally the pacemaker of the frog's heart ? (5) What will happen if you warm or cool the sinus locally ? What will it prove ? (6) Which properties of the heart can be demonstrated on a quiescent heart ? Why cannot these be shown on a beating heart ? (7) What is all or none law ? Why does the heart as a whole obey this law ? Is all or none law contradictory to the staircase phenomenon ? (8) What is the cause of beneficial effect in staircase phenomenon ?

*Experiment No. : 2.10***PERFUSION OF THE FROG'S HEART***
EFFECT OF IONS

Prepare the following solutions for use in this experiment :

1. 0.6 per cent NaCl solution.
2. 1.0 ml of 1.0 per cent CaCl₂ made to 100 ml with solution 1.
3. 1.0 ml of 1.0 per cent CaCl₂ plus 0.75 ml of 1.0 per cent KCl made up to 100 ml with solution 1.

Procedure. (1) Expose the heart of a frog, pass a ligature thread around the sinus and tie a loose knot. Make a small slit in the sinus and introduce a Syme's cannula into it and tie a tight knot around the cannula to prevent the ligature from slipping. (2) Lift the heart out of the body along with the cannula. Fit a Starling's heart lever, upside down, on a stand and fix the Syme's cannula in a clamp directly above the heart lever. Push the pin of the heart lever through the apex of the ventricle and make the necessary adjustments. The contraction of the heart pulls the lever up while the spring of the lever assembly brings the writing lever to the mean horizontal position. Connect the sidearm of the cannula to a burette or a pressure bottle containing Ringer solution.

(a) Perfuse the heart with Ringer and record the cardiac activity.

(b) Replace the Ringer with solution 1 (0.6 per cent NaCl). Note that after a few beats the contractions become smaller and the heart may stop in diastole.

(c) Remove solution 1 and replace it with solution 2 (+Ca²⁺). As the heart is perfused with this it starts beating but over a time the relaxations become less and less, the heart stopping finally in systole.

*Group experiment

(d) Replace solution 2 by solution 3 ($+K^+$). The heart resumes normal contractions and continues to beat for a long time.

This preparation may also be used for the study of effects of temperature and drugs on the heart.

Discussion. Ringer found in 1883, that sodium salts contributed the necessary osmotic conditions for the heart muscle and also maintained the excitable properties of the muscle membrane. Perfusion with sodium salts alone produces weakening and then failure of the beats. Addition of calcium ions restores the contractions. Addition of potassium to the perfusate facilitates the relaxation of the abnormally contracted muscle. The student should consult books regarding the role of sodium, potassium, and calcium in the generation and propagation of the cardiac action potentials and the excitation-contraction coupling.

Experiment No. : 2.11

**PERFUSION OF THE FROG'S
BLOOD VESSELS***
EFFECT OF DRUGS

Procedrue. (1) Stun a frog and destroy the cerebral hemispheres as before but do not destroy the spinal cord. Widen the slit where the needle was passed so that the upper end of the spinal cord is exposed. (2) Expose the heart as before and pass a hread behind one of the aortae and tie a loose knot. Give a nick in the aorta with a fine scissors and introduce a cannula, pointing down-stream into it. Tie the cannula firmly

*Group experiment

in position ensuring that the ligature does not slip. Ligate the other aorta, and make a good-sized cut in the sinus for the exit of the perfusate. (3) Suspend the animal from its lower jaw from a clamp fitted to a stand. Fill the cannula with Ringer with the help of a long-nozzled dropper to drive out the air, and connect it to a pressure bottle containing Ringer. Raise the bottle to about 40 cm above the cannula to provide a pressure head. Collect the fluid draining out from the cut in the sinus and off the frog's legs into a measuring cylinder. The outflow is tinged red in the beginning but becomes clear as the blood is washed out of the circulatory tree. (5) Record the perfusion rate either as drops per half minute or the volume recorded. When the perfusion rate stabilises, perform the following experiments :

(a) Apply one electrode to the spinal cord and the other to the tissues around it. Stimulate with repeated Faradic shocks, measuring the outflow at the same time.

(b) Allow the outflow to become constant, and inject 0.5 ml of 1 in 10,000 solution of adrenalin with a syringe into the rubber tube just above the cannula. Note the decrease in the outflow.

(c) Perfuse with Ringer to stabilize the rate of outflow and inject 0.5 ml of 1 in 100,000 acetylcholine. Record the effect of this drug on the rate of outflow.

As the experiment proceeds the fluid will escape from the blood vessels into the tissues which become tense and swollen i.e. oedematous. The perfusion rate gradually declines due to the external pressure of the fluid on blood vessels and the animal preparation becomes unfit for the demonstration of drugs on blood vessels. This fact should be taken into consideration while interpreting the results of these experiments.

(d) Pass a needle down the spinal canal and destroy the spinal cord. Note the effect of this on the rate of outflow. Inject adrenalin once again and see if it still has a vasoconstrictor effect.

Discussion. Stimulation of the spinal cord produces constriction of blood vessels and this reduces the outflow. Adrenalin is a potent vasoconstrictor agent and it reduces the outflow appreciably. It has the same effect after the spinal cord has been destroyed showing thereby that adrenalin causes vasoconstriction by a direct action on the blood vessels. Acetylcholine produces vasodilatation resulting in increased outflow. Destruction of the spinal cord increases the outflow due to the removal of the tonic vasoconstrictor influence of the sympathetics.

Experiments on The Frog's Nervous System

Experiment No. : 3.1
SPINAL FROG

Observe the behaviour of the intact frog placed on a frog board. The head is well raised on the forelimbs, the hind limbs are flexed, and respiratory movements are present. It responds to various stimuli (e.g., pricking with a needle) by jumping or crawling away. Put it on its back; it rights itself and assumes the normal posture. Put it in a basin full of water ; it swims.

Open the skull in the midline upto the attachment of the forelimbs to the trunk (line AB—Fig. 3.1). Remove the brain carefully and stop the bleeding with cotton swabs. Another method to make a spinal preparation is to cut off the animal's head along the line CD (Fig. 3.1). Use a bone cutter or a stout pair of scissors for this operation, placing the blades across the frog's mouth.

(a) Spinal shock : Put the animal back on the frog board and note that there are no movements. Pinch the skin with a sharp forceps—there is no reflex response of any kind. This state of depression of activity, accompanied by complete paralysis, is called *spinal shock*. The duration of spinal shock varies

in different species—from a few minutes in the frog, a few hours in the dog, to a few weeks in man. The cause of spinal shock is believed to be the sudden interruption of influences descending from the brain to the spinal cord.

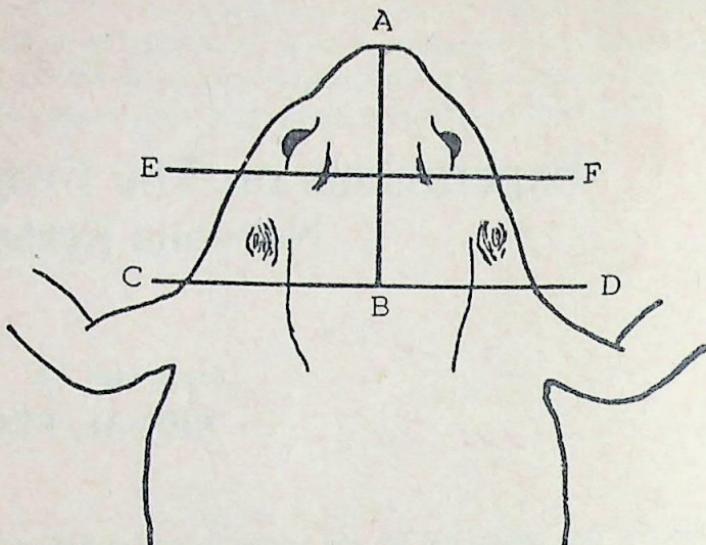


Fig. 3.1 : Lines as incisions to prepare a spinal and a decerebrate frog.

(b) **Stage of reflex activity :** The state of spinal shock disappears soon and the legs are now drawn well upto the body. However, the posture is quite different from the normal. There are no respiratory movements. The frog does not make any 'spontaneous' movements i.e., it does not crawl or jump. Some reflexes can, however, be demonstrated.

Draw down one limb ; it is quickly drawn up. Suspend the animal from its lower jaw on a stand. Pinch one and then the other leg—the animal always withdraws the stimulated leg. Place a small piece of filter paper soaked in sulphuric acid on any part of the back; the animal makes co-ordinated movements with a leg to wipe away the irritating paper. If this limb is held, the animal uses the other leg. The latent period of the movements can be noted ; it will depend on the strength of the stimulus and the irritability of the spinal cord.

(c) **Irradiation of reflexes** : Pinch one toe gently ; there is only a slight reflex movement of that limb. Pinch the toe with greater and greater force, the movements increase accordingly. If the stimulus is still stronger, the other limb, and even the whole animal, may exhibit movements.

(d) **Summation** : Stimulate a toe with weak single induction shocks. There is no response. If these stimuli are applied repeatedly, summation may occur resulting in a reflex movement.

(e) **Measurement of reflex time** : Immerse the hind limbs of the suspended animal in a beaker full of 0.5% sulphuric acid solution. Note the time from the beginning of the stimulus to the reflex withdrawal of the limbs. Wash off the acid with water.

Put the animal in a basin full of water ; the animal cannot swim and sinks to the bottom. Destroy the spinal cord by passing a pithing needle down the spinal canal. No reflex activity can now be demonstrated.

The spinal cord has both motor and sensory functions. It is organized for stretch and other reflexes and there is a supraspinal control over these. These reflexes are important in the motor activities of the animal. Motor fibres from the higher parts of the brain (upper motor neurons) descend down the cord to terminate on the anterior horn cells (lower motor neurons) and are concerned with 'willed' movements. The ascending tracts of the spinal cord carry various sensory signals to the higher parts of the nervous system. The spinal cord has some visceral functions as well.

*Experiment No. : 3.2***DECEREBRATE FROG**

Cut off the head of the frog along the line EF as shown in figure 3.1. After the shock has passed off, place the animal on the frog board and make the following observations :

(a) The attitude of the frog is normal, the head being well raised on the forelimbs. The respiration is almost normal. The animal remains motionless (i.e., there are no spontaneous movements) unless an external stimulus is applied. Some occasional little movements may be seen ; these are due to stimuli arising in the wound.

(b) Pinch some part of the animal with a sharp forceps ; the frog makes complex movements and may jump or crawl away. Place it on its back; it immediately turns over and rights itself. Throw it into a basin of water, the animal swims like a normal frog (the spinal frog sinks).

(c) Place the frog on one end of the board and tilt the board; the animal crawls up the inclined surface to the other end. Tilt the board the other way ; the frog turns and crawls back to its original position.

The effects of decerebration differ in different animals. In the frog, spontaneous or 'willed' movements are, of course, absent but otherwise the animal can crawl, jump, and show other complicated movements in response to external stimuli. It also restores its posture when it is disturbed. In the higher animals, cats for example, decerebration is achieved by cutting the brain stem between the superior and inferior colliculi. Marked rigidity of muscles is seen as soon as the animal comes out of anaesthesia. This state has been called *decerebrate rigidity*. Righting reflexes are absent and the body temperature is not maintained. The decerebrate rigidity is primarily the result of facilitation of the spinal stretch reflexes by impulses descending from the brain stem reticular formation.

SECTION II

EXPERIMENTS ON LABORATORY MAMMALS

Introduction

The practical work undertaken by the students so far illustrates some of the fundamental principles of physiology. All the work in the field of Biology are held together by the important fact that these phenomena constitute parts of a single whole, the living organism. The methods of approach may be quite dissimilar but there is an ultimate unity in the object attacked.

Physiology has developed mainly on the basis of a study of the higher animals including man. The facts accumulated over many decades of extensive experimentation relating predominantly to the mammals, most often the laboratory animals such as rabbits, dogs cats and monkeys etc., have proved to be a major source of our knowledge of the human body. The material provided by such animal experimentation is of inestimable value. Of course, some corrections, which are always indispensable, must be taken into consideration while extrapolating these results to the functions in the human body.

The experiments included in this section are designed to illustrate only some of the functions in the mammals. Many more experiments are possible, especially with the introduction of sophisticated electronic instruments, but the scope of this book has limited these to only a few.

Mammalian Experiments

Experiment No. : 4.1

PERFUSION OF THE ISOLATED HEART OF RABBIT*

The assembly for the perfusion of the isolated heart of the rabbit has the following components :

(1) Water bath with central glass spiral. The water in the bath is electrically heated and its temperature is so controlled that the perfusate flowing out of the spiral and into the cannula is at a temperature of about 38°C. (2) A reservoir placed on a holder fits on a steel upright to provide a pressure of about 60 mm Hg. (3) The outflow tube has two side arms, one of which accommodates a thermometer, while a mercury manometer is attached to the other. (4) An oxygen tube dips in the outlet chamber of the reservoir for oxygenation of the perfusate. (5) Two light pulleys on mounts are provided to guide the thread from the apex of the heart to a Starling's heart lever.

Locke's Solution

Modified Ringer (also called Ringer-Locke's solution) for the mammalian heart consists of : 0.015 g. per cent NaHCO_3 ; 0.04 g. per cent CaCl_2 ; 0.042 g. per cent KCl ; 0.92 g. per cent NaCl and 0.1 g. per cent of glucose in distilled water.

*Group demonstration.

Procedures. (1) Adjust the tap on the oxygen cylinder so that a steady stream of oxygen bubbles passes through the Locke's fluid in the outlet chamber of the reservoir. Keep two basins of very cold Locke's solution ready for receiving the heart from the animal's body. (2) Kill the rabbit by a blow on the head and place it in a dissection tray. Make a midline incision over the sternum and open the chest quickly by cutting the costal cartilages on each side. Expose the heart by snipping through the pericardium. Cut through the roots of the lungs, vena cavae and the aorta, leaving at least 10 mm of aorta attached to the heart. Remove the heart from the animal's body and plunge it quickly into the basin of cold solution. Keep it immersed and pumping gently all the while with a hand to avoid blood clots and entry of air into the coronary arteries. When it is more or less free from blood, transfer it to the second basin. (3) Remove the heart from the Locke's fluid, and slip the aorta on to the cannula and tie it in with strong ligature, leaving as much of the aorta as possible below the cannula. Increase the flow of Locke's fluid by opening the screw clip. The heart is revived within a short time. The aortic valve prevents the perfusate from entering the left ventricle. (4) Pass a thread through the apex of the ventricle and tie a knot leaving the two ends free. Fix these ends to a rod on one side of the apparatus, pulling the heart slightly to that side. Fasten the pin of the heart lever to the apex of the right ventricle and guide the thread over the two pulleys on to the heart lever. The beating of the heart can now be recorded in the usual manner.

The Locke's fluid enters the coronaries and passes to the venous side of the heart through the cardiac tissue. It emerges through the pulmonary artery at the base of the heart, flowing over it and keeping it moist, and drips down the double thread from where it can be collected and measured. This can be utilised to estimate the coronary flow under different conditions. Electrograms can also be recorded with needle electrodes applied to the surface of the heart. Drugs and hormones are injected with a syringe through the rubber tubing between the heart and the heating coil. Wash out a drug with Locke's fluid before using another.

(a) *Effect of temperature.* Inject Locke's fluid at temperatures between 5°C and 40°C with a syringe into the rubber tube and note the usual effects of high and low temperature on the rate and force of heart beat. (b) Injection of 0.5 ml. of 1 in 10,000 adrenaline produces a marked increase in the rate and force of contraction. (c) Injection of 0.5 ml. of 1 in 100,000 acetylcholine has the opposite effects. When the effect has worn off, inject 1 ml. of 1 in 200 solution of atropine sulphate, and test the effect of acetylcholine after atropinization. It is seen that atropine abolishes the inhibitory effect (muscarinic) of this drug. (d) Study the effects of noradrenalin ; posterior pituitary extract and other drugs and hormones as desired. (e) Stop the oxygen supply to the Lock's fluid ; the heart beat continues for some time but soon the contractions become weak. The heart may stop in diastole due to lack of oxygen if its supply is not restored.

Discussion. The coronary arterioles contain both alpha-adrenergic and beta-adrenergic receptors, which mediate vasoconstriction and vasodilatation respectively. Stimulation of the sympathetic nerves to the heart, and injection of adrenalin and noradrenalin increase the rate and force of cardiac contraction. The concomitant increase in the coronary flow may be secondary to the action of local metabolites acting on the coronary vessels, and the rise in aortic pressure.

Note. The effect of various ions on the cardiac muscle of rabbit can also be studied by the use of appropriate solutions as employed in the experiments on isolated heart of the frog.

*Experiment No. : 4.2***EXPERIMENTS ON THE
ANESTHETIZED DOG*****Apparatus**

A long-paper electric kymograph (Brodie-Starling) is fitted on a table with assemblies for a continuous recording of blood pressure, respiratory movements, event marker, time signal marker and a stimulating unit. The paper moves over two cylinders and a flat surface is provided for convenience of entering the data on the smoked paper while the experiment is on. Tuning controls are provided for adjusting the speed of the moving paper in addition to press-button controls for stimulation and event marking.

1. **Manometer.** It is a bent-U tube containing mercury. A float with a curved under-surface and a light-weight capillary carrying a writing point is fitted in one limb of the U tube. The other limb has two side-arms with a three-way stop cock fitted at the level of the lower side arm. A manometric slide-adjustable scale is provided for the recording of blood pressure. The upper side arm is connected to a pressure tube which carries an arterial cannula at its end.

2. **Pressure bottle.** It contains 10 per cent sodium citrate solution and has two tight-fitting rubber corks, one each at the top and the exit. The glass tube fitted in the bottle is kept above the fluid level and carries an air bulb. The exit tube connects the pressure bottle to the upper side-arm of the manometer.

3. **Instrument tray.** The following instruments and drugs will be needed ; Scalpel, scissors, artery forceps, bull-dog clamp, syringes and needles, retractors, tracheal cannula, Francis-Francois arterial cannula (the bulb of the arterial cannula has a side arm carrying a rubber tube and a screw clamp and is used

*Group demonstration.

for removing blood clots if any), venous cannula, gauze pieces and cotton swabs wetted with saline ; adrenalin, acetylcholine, atropine solution and nembutal.

4. Operation table. It has cleats provided on the sides for fastening the limbs of the animal. The stainless steel top is in two halves sloping towards the centre and is heated by a set of electric lamps fitted under it. Blood and other fluids flow towards the centre of the table from where these drip into a removable drain pipe. The excised tissues, used swabs etc. are collected in an enamel bucket for proper disposal later. A dog holder is fitted at the head of the operation table and a steel upright carries a swing tray for placing the dissecting instruments.

Selection and Preparation of the Animal

Do not use large animals : a dog weighing between 7 and 10 kg is an ideal one for most purposes. Keep the animal in quarantine for 10 days. Never frighten, annoy, give pain to the animal or otherwise mishandle it. It is not *only* inhuman, *but* also likely to spoil the experiment. If scratched or bitten by the dog, wash with alcohol and apply carbolic acid. Do not feed the animal for 6 to 8 hours before the experiment.

Anaesthesia

A volatile (ether) or a non-volatile anaesthetic may be used. Nembutal (pentobarbitone sodium), in doses of 30 mg. per kg. given intraperitoneally produces a smooth and uniform depth of anaesthesia under most conditions. Small doses can be repeated during the experiment if necessary. If using ether, keep a watch on the corneal reflex and the pupillary size. The disadvantage of ether is in that it has to be administered continuously and a careful watch has to be kept on the vital signs.

The other anaesthetics which may be used are : Urethane=1 mg per g as a 25 per cent solution and Chloralose=80--100 mg per kg as a 10 per cent solution in warm saline.

Operation

While an assistant holds the head and the limbs of the animal, inject the appropriate amount of nembutal, dissolved

in 10 ml of warm saline, intraperitoneally. When the legs are relaxed and the corneal reflex very sluggish or has just ceased, the animal is ready for operation. Transfer the animal to the operation table and fasten its limbs to the cleats with pieces of strong cord. Apply a clamp to the tongue, draw it out and leave the clamp in position. This prevents the flaccid tongue from falling back into the pharynx and causing respiratory obstruction.

(a) **Venous cannulation.** Expose the femoral vein, artery and nerve in the thigh. Isolate the femoral vein for a length of one inch and tie a ligature on it away from the heart. Give a nick in the vein and fix a venous cannula into it, connecting it to a burette containing normal saline. The venous route is employed for injecting drugs and saline into the circulation.

(b) **Tracheostomy.** (1) Clip the hair from over the trachea. Give a 3 inch long midline cut with the full edge of a sharp scalpel, below the larynx, through the skin and fascia. Press the surface with wet gauze to prevent oozing of blood. Catch any bleeding vessels with artery forceps and ligate these if necessary. Separate the sternohyoid muscles and expose the trachea by blunt dissection, using retractors if needed. The carotid sheaths containing the carotid artery and the vagosympathetic nerve lie about an inch on either side of trachea. Isolate the carotid sheaths on both sides for a length of about 2-3 inches and place loose ligatures around both. Lift up the trachea and pass a piece of strong cord underneath it. Make a V-shaped cut in the trachea with a scissors and insert a tracheal cannula. Tie it firmly in position and open its vent slit. This is used for artificial respiration if the chest is opened for the observation and recording of cardiac activity. (2) For the recording of respiratory movements apply a stethograph on the chest and connect it to a Brodie's tambour. Make necessary adjustments to get good excursions of the writing lever.

(c) **Recording of arterial pressure.** In 1733, the Rev. Stephen Hales, an English clergyman, inserted a brass cannula into the femoral artery of a mare and connected it to a 9 feet long glass tube. On releasing the ligature on the artery the blood rose to

a height of 8 feet 3 inches and oscillated above and below this level. The blood column rose only 12 inches when the cannula was inserted in the femoral vein. This was the first instance of a quantitative determination of blood pressure.

(1) Create a pressure of about 100 mm Hg in the manometer by raising the pressure bottle or pumping air into it. Open the screw clip on the tube leading to the arterial cannula to drive out the air from the pressure recording system and to fill it with the anticoagulant fluid. (2) Open the carotid sheath and isolate the artery from the nerve for a length of 2 inches and place two loose ligatures around each. (3) Ligate the artery tightly as far removed from the heart as possible and place a bull-dog clamp on the artery as close to the heart as possible. Place a strong thread beneath the artery between the ligature and the clamp. (4) Lift up the artery swollen with blood and place a finger beneath it. Make a V-shaped cut in the wall of the artery with a small scissors close to the tight ligature and slip the wetted arterial cannula into it; tie it securely in place, fixing the ends of the thread around the side-arm of the cannula to prevent slipping of ligature. (5) Remove the screw clamp from the rubber tube so that the blood pressure is transmitted to the manometer. Adjust the position of the weighted thread to bring the writing point of the float in light contact with the smoked surface. Record the respiratory movements above the blood pressure tracing. (6) Expose the artery and the nerve on the opposite side and place loose ligatures around each. (7) Calibrate the recording surface for blood pressure tracing with the help of the manometric scale before the start of the experiment.

The blood pressure tracing. The mercury manometer does not faithfully reproduce the usual fluctuations in blood pressure associated with the cardiac cycle because of its high inertia and low natural frequency. The fluctuations in pressure are damped; the high values are less and the lower values greater than the real values. The oscillations of the mercury with each beat of the heart are, therefore, small. The blood pressure so recorded gives an indication of only the mean pressure. As the flow in the artery is interrupted the pressure recorded is an end-on

pressure). All the energy of flow is converted into pressure energy. If the pressure were recorded from the side-arm of a T-tube inserted in an artery, the recorded 'side pressure' would be less than the end pressure by the kinetic energy of flow.

Usually three kinds of oscillations are recorded :

(a) Those caused by the cardiac contractions which send additional amounts of blood at each systole—the *heart waves*. These are super-imposed on the respiratory waves.

(b) *Respiratory waves or Traube--Hering waves*. These are variations in blood pressure synchronous with the movements of respiration and show an increase during inspiration and a decrease during expiration. The waves may be quite large if the respiration is slow.

(c) *Meyer's waves*. These are fluctuations in blood pressure over long periods and are caused by periodic changes in the tone of blood vessels. These are not always well marked.

EXPERIMENTS

1. **Stimulation of vagosympathetic trunk.** The first instance of inhibition discovered in Physiology was in 1845 when the Weber brothers found that the stimulation of vagus decreased the heart rate whereas a stronger stimulation stopped it. In the heart the vagal fibres terminate on some clusters of neurones (nervous nuclei of Dogiel, Remak, Bidder and Ludwig). These masses are located in the sinuatrial and atrio-ventricular nodes. The postganglionic fibres pass in the coronary plexuses to the atria, the A-V bundle and the base of the ventricle. The fibres in the right vagus end mainly at the S-A node and in the left chiefly around the A-V node.

The sympathetic fibres arise in the 2nd and 3rd thoracic segments of the spinal cord and pass in the anterior roots as myelinated fibres to the cervical sympathetic ganglia where they synapse. From here the postganglionic fibres begin and pass to the cardiac plexuses where they intermingle with the vagal fibres to end in the heart muscle.

Tone of the vagal centre. A prolonged excitation which is not attended by fatigue is called tone. Impulses are constantly passing down the vagus nerves to the heart to exert a sustained inhibitory action on it. It is partly through variation in this vagal tone that changes in heart rate are produced according to the needs of the body. The cause of the vagal tone is the negative feedback from the arterial baroreceptors to the cardioinhibitory centre in the medulla.

Stimulation of vagi. Stimulate the vagi with weaker and stronger Faradic shocks. The main effect of stimulation of the right vagus is a slowing of heart rate and a fall in blood pressure. Stimulation of the left vagus depresses mainly the A-V conducting system. Most parts of the heart, however, receive vagal fibres from both sides so that the effects of their stimulation are more or less identical viz. slowing and decrease in the force of contraction of heart and a fall in blood pressure. Influences on the rate of heart beat are called chronotropic effects (Greek *chronos*=time), whereas the influences on the force of contraction are named inotropic effects (Greek *inos*=force).

With the slower beating of the ventricle, the diastolic period is prolonged, the ventricular filling is more and the stroke volume is increased. These are reflected in wide swings in the oscillations of the mercury column. If the stimulation is continued, the ventricles resume beating and escape the inhibitory effect of vagus. (With an appropriate method it can be shown that there is no 'escape' of atria). The phenomenon of vagal escape indicates that the ventricles, when deprived of impulses from the atria, can start an independent, though slow, rhythm. The vagal escape perhaps also suggests that the vagus has very little direct effect on the ventricular activity.

2. **Adrenalin.** Inject 1 ml of 1 in 10,000 adrenalin solution into the femoral vein. There is an immediate but small and transient fall of arterial pressure, which is at once followed by a rise. This lasts for only a few minutes and is not sustained. This drug produces constriction in the cutaneous, pulmonary and visceral vessels but dilatation of the skeletal and hepatic

vessels. The net result is a somewhat lowering of the total peripheral resistance. The increased cardiac output must obviously be responsible for the rise in blood pressure. With the rise of pressure the respiration is reflexly arrested ; this has been called *adrenalin apnoea*. It will be a profitable exercise to study the effects of different doses of adrenalin on blood pressure.

3. Injection of noradrenalin is not followed by the initial fall in arterial pressure but the elevation in pressure is greater and more sustained. The mean pressure rises despite reduction in cardiac output by the baroreceptor mechanism. It is thus a general vasoconstrictor.

4. Injection of posterior pituitary extract produces a rise in pressure by its direct vasoconstrictor action on blood vessels. (The amount of vasopressin secreted in the body has no physiological role in the regulation of blood pressure).

5. **Acetylcholine.** Inject 1 ml of 1 in 100,000 of the drug into the venous system. There is a marked fall in pressure resulting from an extensive dilatation of the arterioles, the slowing of the heart also contributing to it.

6. **Carotid sinus.** Follow the carotid artery to its division by careful dissection. Injection of normal saline into the carotid sinus with a syringe produces a fall in blood pressure. This is due to the stimulation of the stretch receptors located in the tunica adventitia, which send inhibitory impulses to the medulla via the nerve of Hering, a branch of glossopharyngeal. Pressure in the sinus can be lowered by compressing the common carotid artery below the sinus ; the result being a rise in the blood pressure. The activity of the carotid sinus, however, can be studied best when the blood pressure is recorded in the femoral artery and the experiments done on an isolated sinus.

7. **Section of vagi.** Cut the right vagus between the two ligatures and note the effect on blood pressure and respiration. Cut the vagus on the opposite side ; the heart accelerates one and a half to two times (loss of vagal tone) and there is a rise in blood pressure. The respiratory movements become slower and deeper, the effect being smaller when only one vagus is cut.

8. Stimulation of the peripheral ends of the cut vagi produces a fall in blood pressure and inhibition of the heart. The respiration is unaffected.

9. Stimulation of the central ends of the cut vagi stops the respiration. Study the effects on blood pressure in each case.

10. **Asphyxia.** Apply a clamp on the trachea to produce asphyxia i.e., hypoxia and hypercapnia. There is a marked stimulation of respiration and the animal makes violent respiratory efforts. The CO₂ has a strong stimulatory effect on the vasomotor centre which elevates the blood pressure and the heart rate. The animal may be revived at this stage but if the asphyxia is maintained, the heart slows down and the blood pressure falls. There is generalised twitching of muscles and finally the efforts at respiration fail. Ventricular fibrillation and cardiac arrest occur in about 4 to 5 minutes.

11. **Haemorrhage.** Introduce a cannula, bearing a short length of rubber tube with a clip, in a femoral artery for removing blood into a measuring cylinder. Remove 25 ml portions of blood at timed intervals and record the effects on blood pressure and respiration.

The effects of loss of blood depend on the amount of blood removed and on the rapidity of bleeding. Removal of small amounts does not lower the mean arterial pressure though the decreased blood volume may reduce the cardiac output. The following mechanisms tend to maintain the blood pressure.

(1) *Blood depots.* The entire amount of blood in the vascular system does not participate equally in circulation. The vascular system of the spleen in the dog may store 15 to 20 per cent of the animal's blood (in man the storage function is very little, if any). Contraction of the smooth muscle of the spleen thus "pours" blood into the circulation. The vasculature of skin, liver and lungs has also some blood stores which are called upon when needed. (2) Almost 50-60 per cent of the blood is in the venous system at any one moment. Venoconstriction, therefore, provides a valuable reserve of blood for the venous return to the heart. (3) Reflex vasoconstriction of arterioles,

in addition to increasing the peripheral resistance, causes a fall of hydrostatic pressure in the capillaries thus reducing the tissue fluid formation.

With the removal of 10-15 per cent of the circulating blood, a significant fall in blood pressure is largely prevented by intense constriction of arteries and veins resulting from (a) reflexly mediated sympathetic vasoconstrictor impulses and (b) discharge of adrenal medullary hormones—adrenalin and noradrenalin into the circulation.

Circulation fluid volume. There is evidence that the blood volume, and so the fullness of the vascular system is monitored by low pressure receptors in the atria and the big veins. These volume receptors reflexly affect the secretion of ADH from the posterior pituitary.

With a decrease in blood volume, the venous return and the cardiac output decrease and as the defence mechanisms offered by baroreceptor reflexes fail, the blood pressure shows a considerable fall. The stagnant hypoxia stimulates the carotid and aortic body chemoreceptors which produces rapid and shallow breathing.

Determine the effects of saline infusions at various stages of haemorrhage.

Note : Keep a watch on the body temperature of the animal throughout the experiment with a rectal thermometer.

Killing and Disposal of the Animal

After the conclusion of the experiments the animal is killed by injecting enough of ether into the femoral vein.

Experiment No. : 4.3

RECORDING OF INTESTINAL MOVEMENTS OF RABBIT IN VITRO*

Apparatus

Long paper extension electric kymograph.

Student's jar bath : The outer container, usually made of perspex, contains tap water at 37°C and the temperature can be maintained with a heating element. A central glass vessel of 100 ml. capacity with an outlet at its lower end is fitted at the bottom of the container. A hollow glass tube with a hook at its lower end is clamped to a pillar by a boss head so that it can be lowered into the central vessel which contains Tyrode's solution at 37°C. The pillar also carries a steel capillary lever with a frontal writing point giving a magnification of about three or four times.

A more sophisticated apparatus has a thermostatically controlled heating element and a glass warming coil. The coil lies in the outer bath with one end connected to the side arm of the central vessel and the other to a bottle containing Tyrode's solution. The inner or central vessel is fitted into a water-proof seal at the bottom of the bath with the outflow tube projecting out for the draining off of the Tyrode's fluid from the central vessel. An electric stirrer is usually fitted to one edge of the perspex bath. The Tyrode from the bottle passes through the glass coil and can thus replace the solution in the inner vessel at the correct temperature, whenever desired. Oxygen cylinder, Rubber tubes, Metal screw clamps. Instrument tray with scalpel, scissors, forceps, needles, thread and syringes. Drugs and hormones : 1 in 10,000 adrenalin ; 1 in 100,000 acetylcholine ; 1 per cent atropine sulphate ; 1 in 10,000 histamine hydrochloride ; 2 per cent barium chloride ; Mepyramine and Oxytocin.

*Group experiment

Tyrode's solution. 0.8 g NaCl ; 0.02 g. KCl ; 0.02 g. CaCl₂ ; 0.01 g. MgCl₂ ; 0.1 g. NaHCO₃ ; 0.1 g. glucose ; 0.005 g. NaH₂PO₄ and distilled water to 100 ml.

Procedure. (1) Kill a rabbit and open its abdomen with scissors. Remove gently, and without stretching the gut, a few 3-4 cm long portions of jejunum, minus mesentery, and transfer these to a large petri dish containing warm Tyrode's solution. Oxygenate the solution from time to time. These pieces will show spontaneous worm-like movements in due course. (2) Fill the inner glass vessel with Tyrode at 37°C and switch on the electric heater and stirrer. (3) Pass a threaded needle through the wall of a piece of intestine from inside to outside and make a small loop, leaving the thread in place. Pass another threaded needle through the other end, tie a knot and leave about 15-20 cm of thread. *Do not close off the lumen of the gut.* (4) Pass the loop over the hook of the oxygen tube and tie a knot. Lower the glass tube, along with the piece of intestine into the Tyrode solution in the inner vessel and fix it in the adjustable hook grip to keep it in position. (5) Tie the long thread to the lever near its end and apply a bit of plasticine to keep the thread in position and to act as a light counterweight. Adjust the lever so that the gut is slightly stretched. Connect the oxygen tube to a cylinder so that 2-3 bubbles of oxygen pass through the Tyrode per second. By this time the movements would have become fairly regular. The writing lever is pulled down by contraction and as the fulcrum is near the long thread the writing-point moves up. (6) Bring the writing point in gentle contact with a slow moving cylinder and record the rhythmic pendular movements. The double-pronged writing lever remains in contract with the smoked surface without much friction.

Experiments. (a) Add 1 ml of adrenalin to the Tyrode solution and indicate this point with an arrow. This drug causes a marked decrease of tone and a decrease or stoppage of the movements. The movements return after the Tyrode is replaced. (b) Add 0.5 ml of acetylcholine solution. There is not only an increase in the tone i.e., the tracing moves up to a higher level, but the rate and amplitude of contractions also increase. After

atropinization, acetylcholine has no effect. (c) Addition of histamine increases the muscle tone. Its effect can be blocked by pretreatment of the gut with mepyramine. (d) Barium chloride produces a strong contraction due to the direct effect of barium ions on the smooth muscle. (e) Oxytocin causes stimulation by a direct action on the smooth musculature.

Discussion. The strip of intestine shows fairly rhythmic contractions and relaxations. These movements are best recorded from the jejunum. Two features of the smooth muscle of intestine are well illustrated-namely, the tone and rhythmic contractions.

The smooth muscle is self-excitatory. There is a basic rhythm in the intact animal which can initiate action potentials. Two types of functional movements occur ; mixing movements or segmentation contractions and propulsive movements or peristaltic waves. Both can occur in the absence of extrinsic innervation, but an intact myenteric plexus is essential. Usually the peristaltic movement is superimposed on the rhythmic segmenting contractions in such a manner that the two are present simultaneously. The peristaltic contraction is manifested as an increase in the level of tone of the intestinal musculature, without any interruption in the rhythm of the segmenting contractions.

The basic contractile rhythm of the upper part of the gut usually shows a higher degree of activity than the lower part. Available evidence indicates that greater number of peristaltic impulses are generated in the upper portion of the gut than the distal regions, resulting in the travelling down of peristaltic waves. The upper portion in this sense acts as a pacemaker for the contractile activity of the gut. This is the basis of the gradient theory for forward propulsion.

The visceral smooth muscle has an unstable membrane potential and sine wave-like fluctuations, only a few millivolts in magnitude, are superimposed on this. In addition, there are spikes of variable amplitude. Because of the continuous activity, the relation of electrical to mechanical events is difficult to study. However, the muscle tissue starts contracting about 200 m sec

after the start of the spike and continues 150 m sec after the spike is over. Thus the excitation contraction coupling is a very slow process. Furthermore, the visceral muscle contracts when stretched in the absence of any extrinsic nerves. The intestinal muscle is innervated both by adrenergic and cholinergic nerves. Activity in the latter enhances the intrinsic activity of the smooth muscle while activity in the former decreases it.

Serotonin and substance *P* are believed to be the chemical mediators at the junctions between the neurones of the myenteric plexus.

SECTION III

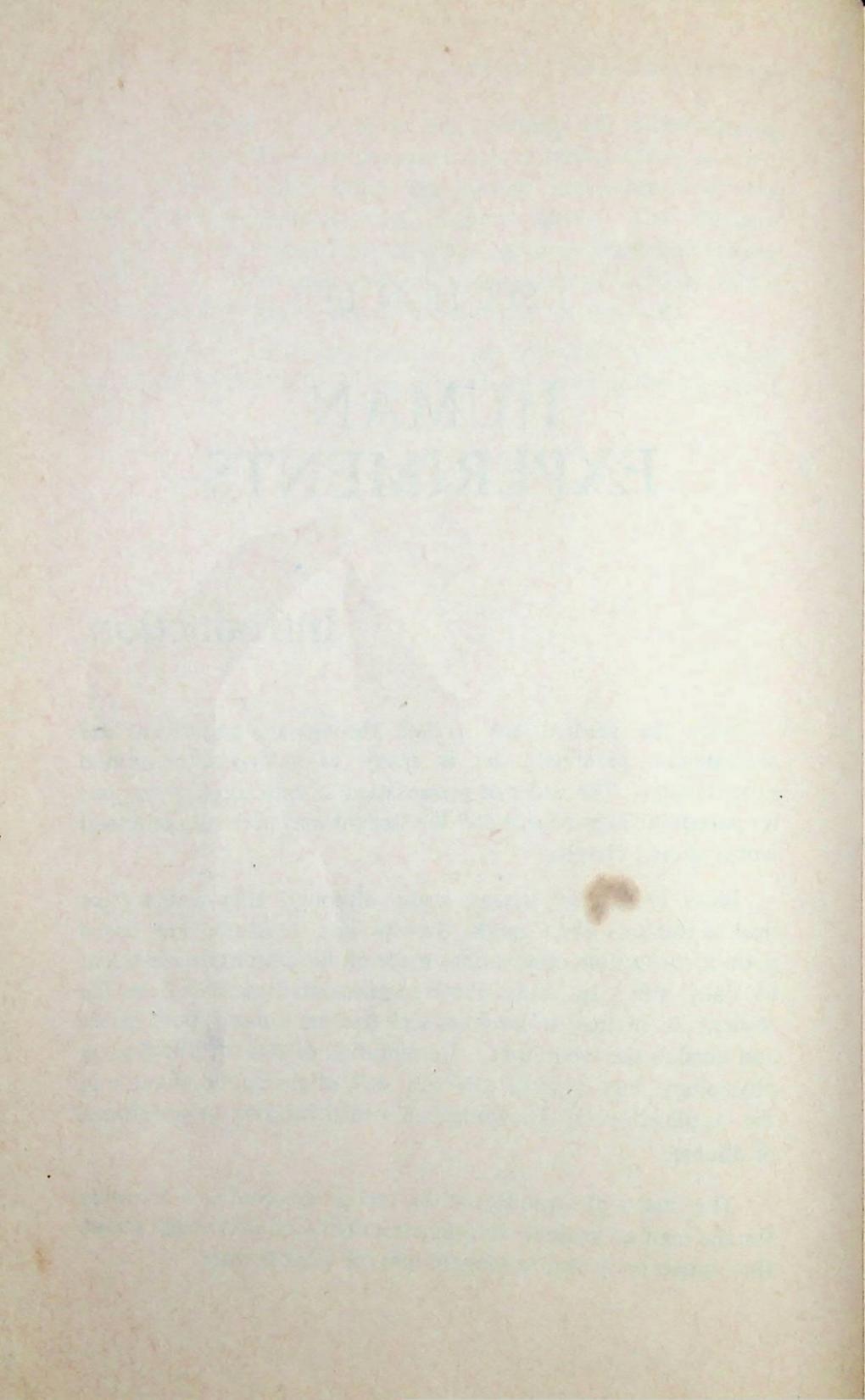
HUMAN EXPERIMENTS

Introduction

After the student has worked through the amphibian and mammalian practicals, he is ready to undertake the human experiments. The order of presentation of various chapters has no particular significance and the student may start the practical work in any chapter.

Ideas about the human body, although they owe a great deal to thinkers like Charak, Susruta and Aristotle, are based upon innumerable observations made on the phenomena incident to daily life. In many short experiments described here, the student is invited to perform such tests on himself, both inside and outside the laboratory. Consultation of standard books on physiology and clinical methods will, of course, be of value in the application of knowledge of normal function to conditions of disease.

The material included in this section is meant as a stimulus for the medical students to gain some first-hand knowledge about the human body and to prepare him for clinical work.



Haematology

INTRODUCTION

Blood consists of formed elements—the red blood cells, white blood cells and platelets which are suspended in a fluid medium. These cells have a variety of functions, like carriage of gases, phagocytosis, protecting the body against infections, immunological responses, and controlling the bodily responses to blood vessel injury.

The average blood volume in an adult is about 5 liters, 43-45 per cent of which is constituted by red cells. The blood volume is kept remarkably constant under various conditions by a series of compensatory mechanisms.

The experiments described in this chapter are carried out as routine laboratory tests in hospitals and clinics for diagnostic as well as prognostic purposes in disorders of blood. Use of the microscope, collection of blood samples (venous and capillary), the correct procedure of using a diluting pipette for cell counts, description of a haemocytometer and how to charge it with diluted blood, and preparing a blood smear are described in detail in the first few pages. This approach will avoid repetition in the related estimations and calculations and thus make the student's task easier. He must go through these pages with great attention and understanding. He can then, refer back to these if and when required. Procedural details of other determinations are described with each experiment.

Though the student will estimate his own haemoglobin level, cell counts etc., the volunteer's venous blood must only be collected by a staff member who must screen the subject for history of communicable diseases, especially viral hepatitis.

Caution : The volunteer's blood must be regarded (and also that of a patient's) as a potential source of infection and should not be touched.

Experiment No. : 5.1

STUDY OF THE COMPOUND MICROSCOPE

Before using a microscope, the student must familiarise himself with the different parts of the microscope (Fig. 5.1) and how to handle and take its care. A brief description is given below :

- (1) **Base.** This supports the microscope on the working table and is U-shaped to give the microscope maximum stability.
- (2) **Pillars.** Two upright pillars project up from the base and the handle of the microscope is hinged to these.
- (3) **Handle.** It is curved and the microscope can be tilted at the hinged joint, when desired.
- (4) **Body tube.** It is attached to the handle and can be raised or lowered by a rack and pinion arrangement
- (5) **Coarse and fine adjustment screws.** These are employed to bring the object being studied into focus. The body tube can be raised or lowered quickly with the coarse screw, and finer adjustments are then made with the fine adjustment screw for exact focussing. There are two coarse and two fine adjustment screws mounted at the top of the handle by a double-side micrometer mechanism, one pair on either side. If one screw

is rotated, its member on the opposite side also rotates at the same time. It is not logical, therefore, to operate the

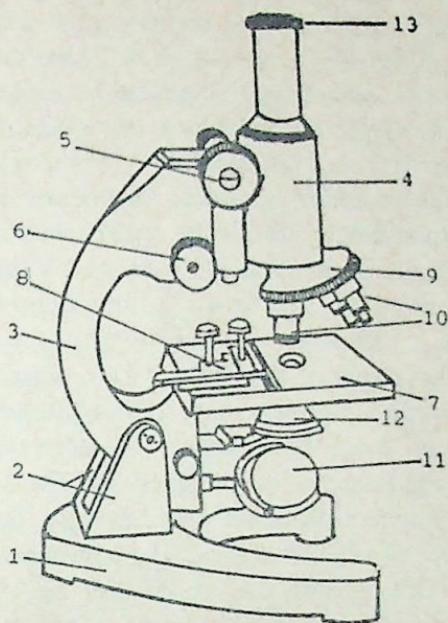


Fig. 5.1 The compound microscope. 1-Base ; 2-Pillars ; 3-Handle : 4-Body tube ; 5-Course adjustment screw ; 6-Fine adjustment screw ; 7-Fixed stage ; 8-Mechanical stage ; 9-Fixed and revolving nose-pieces ; 10-Objective lenses ; 11-Mirror ; 12-Condensor ; 13-Eyepiece

adjustment screws from both sides simultaneously. *Left hand is used both for coarse as well as fine adjustment screws and right hand for manipulating the mechanical stage only.* (6) Fixed stage. This is the square platform, with an aperture in its centre, on which the slide or counting chamber is placed. The converging cone of light passes through the aperture and is directed through the slide into the objective lens of the microscope. (7) Mechanical stage. It is calibrated and fitted on the fixed stage. There is a spring-mounted clip to hold the slide or the counting chamber in position, and two screws for moving these transversely, or forwards and backwards. (8) Nosepiece. The fixed nosepiece is attached to the lower end of the body

tube and the revolving nosepiece is mounted under it. The revolving nosepiece carries the objective lenses of different magnifying powers. (9) **Objective lenses.** (also called objectives). Three objectives, screwed into the revolving nosepiece, are usually provided with the student microscope. The magnifying power of each objective is indicated on it. There are : (a) $10 \times$: *Low-power objective*, magnifies the image 10 times, (b) $40 \times$ or $45 \times$: *High-power objective*, magnifies the image 40 or 45 times and (c) $90 \times$ or $100 \times$; *Oil immersion objective*, magnifies 90 or 100 times. The revolving nosepiece allows any objective to be swung into position below the body tube, the correct position being indicated by a slight "click". In many microscopes, a very low-power objective ($3 \times$ or $4 \times$), the 'scanning' objective, is also available. This helps in adjusting proper illumination of a relatively large area of the slide. The objectives supplied with the microscope these days are manufactured such that if one objective is in focus (low-power for example), the others are more or less in focus; when switched into focussing position ; switching from low to high power requires only fine adjustment. This arrangement of objectives is called *parfocal* arrangement. (10) **The eyepiece.** This fits into the top of the body tube. Most microscopes are provided with two or three eyepieces with magnifying powers of 5, 8, or 10 times, though eyepieces which magnify 6, 15, 20 or 25 times are also available. Pointer eyepieces with movable indicators and 'demonstration eyepieces' are commonly used to help the students in indentifying various objects on the slide. **Total magnification.** The utility of any microscope depends not merely upon its magnifying power, but on its power of resolution i.e.. its ability to improve the details. The resolution is due only to the objective lens, the eyepiece only enlarges the image produced by the objective lens without improving its quality. For practical purposes, it may be remembered that with the ordinary light microscope, the useful magnification is about 1500 times, beyond which the images are not very distinct. Electron microscopes can give very high magnifications. Although the light passes through a complex system of lenses through the microscope, the total magnification obtained can be easily calculated by

multiplying the magnification of the objective with the magnifying power of the eyepiece. For example, with an eyepiece of $10\times$, the total magnification with low-power objective will be 100 times (10×10), with high-power objective, 400 times (10×40) and with the oil immerson objective, it will be 1000 times (10×100). With lower magnification, a larger area can be seen, whereas, with higher magnifications, the field is restricted.

(11) **The mirror and illumination.** The mirror is fitted below the condenser and its purpose is to *reflect* light into the condenser. There are two surfaces (in fact two mirrors), one flat or plain mirror, and the other concave. It can be adjusted in any direction. Plain mirror is used with a distant light source (natural daylight), and concave mirror is used when the source of light is near the microscope. In the former case, parallel rays of light are reflected parallel into the condenser, while the concave mirror converts diverging rays into parallel rays of light. Research microscopes have built-in electric light source.

(12) **The condenser.** This is fitted under the fixed stage and usually has two lenses mounted in a short cylinder. An iris diaphragm is provided to control the amount of light entering the microscope. The parallel beam of light is converged into a cone of light which passes through the aperture of the fixed stage. There is an arrangement of accommodating a blue filter and ground glass (if not already provided with the microscope) which should be used when an artificial source of light is providing the illumination. The condenser can be lowered or raised by a rack and pinion arrangement with the help of a screw.

Use of Objective Lenses

The resolving power of an objective depends on its light gathering capacity or the numerical aperture (NA) as well as the wave length of light. With an oil immersion objective ($100\times$) with a numerical aperture of 1.30, the resolving power is about $0.25\text{ }\mu\text{m}$ or 2500 Angstrom units.

1. *Low-power objective* ($10\times$: NA: 0.25). The students must always start with the low-power lens as the field under view is much larger. Identification of a tissue, or focussing the lines on a counting chamber is much easier when the LP lens is used, *the condenser being moved down and the iris diaphragm adjusted*

so that there is no glare. The aperture (not NA) of this lens is bigger than that of the other objectives.

2. *High-power objective* ($100\times$: NA: 1.65). This is used for a more detailed examination of a particular area which has been brought into the centre of a field by the low-power objective.

3. *Oil-immersion objective* ($100\times$: NA : 1.30). This objective lens can be readily recognised by a black ring etched around it near the lower end. The aperture through which light passes is very small (the size of a pinhead). This lens has to be used with caution, as its focussing position is very close to the slide and there is real danger of making a direct contact with the slide or counting chamber and thus breaking these or causing damage to the lens. As its name implies, the objective has to be immersed in an oil. A thin layer of air is present between the objective and the coverslip. When light passes through a denser medium (glass in this case) into a rarer medium (air) the light rays are refracted away from the normal, thus very little light enters the objective, resulting in a blurred image. Cedar wood oil, which has the same refractive index as that of glass, is therefore used to replace the air and prevent refraction of light. The correct method of using oil-immersion objective is to raise the body tube and swing the lens into position. A drop of cedar wood oil is then placed on the slide and watching from the side, the objective is slowly brought down till it just enters the drop of oil; final focussing being achieved with the fine adjustment screw only.

How to Use the Microscope

The first rule in studying a slide is to examine it with the naked eye. This must always precede the microscopic examination.

Place the microscope on the working table, in an upright position, and put the slide or chamber on the fixed stage. The correct procedure is to start always with the low-power objective lens. Adjust the selected mirror for optimal illumination. Raise the body tube with the coarse screw, and looking from the side, bring the objective down to about 1.0 cm above the slide. Look

into the eyepiece and gently raise the body tube till the object comes into focus, but if it does not, *repeat the whole procedure again*. You can further clarify the image by using the fine adjustment screw. *Never* start the focussing process by bringing down the body tube from a height while looking into the microscope, because you might miss the focussing point and continue moving the tube down, thereby breaking or damaging the slide or the chamber. Although you are using one eye with the monocular microscope, do not close the other eye, but learn to ignore its image as this puts unnecessary strain on the eye. With practice, your eyes will adjust to the conditions and you will be able to work for long periods without tiring.

Note. If there are any smudges, dust particles or threads visible when the slide is in focus, rotate the eyepiece to confirm whether these are on the slide or on the eyepiece. If these move with the eyepiece, take out the eyepiece, unscrew the lenses and clean these carefully.

Other types of microscopes are :

1. *Dissection microscope.* It is a binocular microscope and is used for microdissection studies under magnification.
2. *Binocular microscope.* This is used when one has to work for long periods and in research work.
3. *Phase contrast and interference microscope.*
4. *Fluorescence microscope.*
5. *Electron microscope.* It uses a beam of electrons instead of light rays. The magnified image is visible on a fluorescent screen and can be recorded on a photographic film. The magnification obtained is very high as the wavelength of a stream of high speed electrons is so short that the resolving power of this microscope is about 10A° or so. A common practice is to obtain the image on the photographic plate at magnifications of 5,000 to 20,000 times, the negative is then enlarged about 6 to 8 times thus giving a total magnifications of 120,000 x or more. Electromagnetic fields are used in place of lenses.

Precautions. 1. Follow the instructions strictly. Do not keep the microscope near the edge of the table.

2. All traces of cedar wood oil must be removed with a piece of clean flannel. Use xylol to remove oil from the oil-immersion lens.

3. Do not tilt the microscope while counting cells in a chamber or when cedar wood oil is being used. Tilting is hardly ever needed when working for short periods.

4. Always look for foreign particles and different types of artefacts like precipitates, shrinkage, folds etc. in a tissue section.

Questions : (1) When are plain and concave mirrors used ? (2) What is the function of the condenser ? What should be its position when using the low-power objective lens ? (3) How is the total magnification obtained with a particular objective calculated ? (4) Why is (any) oil required to be used when using the oil-immersion lens ? Why is cedar wood oil particularly chosen for this purpose ? (5) Name some other types of microscopes.

Experiment No. : 5.2

COLLECTION OF BLOOD SAMPLES*

Usually 5-6 ml of blood is collected from a vein or an artery for a comprehensive haematological investigation. Capillary blood may be used for haemoglobin estimation, cell counts, blood grouping, bleeding and coagulation time, or for microchemical investigations.

A Venous Blood

Puncturing a vein with a needle attached to a syringe is called *venipuncture*. Use a sterile, dry, 10 ml syringe with a

*Group demonstration.

wide-bore and a short-bevel needle (No. 1 or 2). Narrow and short injection needles are not suitable for this purpose.

Support the arm on the edge of a table. Locate a vein visually (superficial veins are usually slippery ; a vein embedded in the subcutaneous fat is easier to handle) in the antecubital fossa. Clean this area with a swab of cotton dipped in alcohol (rectified spirit or methylated spirit) and allow it to dry. Apply a cloth tourniquet firmly around the upper arm and ask the subject to open and close the fist repeatedly. The veins become engorged with blood. Choose a proper vein and introduce the needle into the skin with a firm and smooth motion. Puncture the vein a few millimetres ahead of the skin puncture site and a little from the side of the vein. (This prevents counterpuncture of the wall of the vein and unnecessary bleeding and haematoma formation after the needle is withdrawn later). Draw the required amount of blood by pulling back on the plunger of the syringe gently but firmly. Release the tourniquet and withdraw the needle after 5-6 ml of blood has been obtained. Put a fresh cotton swab over the skin puncture and ask the subject to flex the arm at the elbow, keeping the swab in position for 1-2 minutes, till the bleeding stops.

Remove the needle from the syringe and expel the blood gently into a prepared container of anticoagulant. Shake the container gently so that the anticoagulant mixes well with the blood and prevents coagulation.

In obese subjects or in very young children, it may sometimes be difficult to locate a good vein in the antecubital area. In such cases, veins on the back of the hand, femoral vein (in infants first locate the femoral artery by its pulsations, the vein lies medial to it. In very young infants blood may have to be collected from the frontal venous sinus in the skull), or jugular vein may be used for obtaining the sample.

Anticoagulants required. 1. Double oxalate mixture. Prepare a solution of potassium oxalate 2 g, ammonium oxalate 3 g, dissolved in 100 ml of distilled water. You can prepare a large number of containers at one time (discarded medicine bottles in the wards or 10 ml test tubes) by placing 0.2 ml of the oxalate

mixture and allowing the water to evaporate on gentle heat in an oven. Add the blood to a container and mix gently, avoiding any foaming. (Do not use ammonium oxalate for non-protein nitrogen or urea determinations). Potassium oxalate alone causes shrinkage of red cells whereas ammonium oxalate increases their volume. It is for this reason that a mixture of these is used.

2. Heparin solution or powder may be used as required.

3. Salts of ethylenediamine tetracetic acid (EDTA-'Sequestrene') can be used. The salts prevent coagulation by removing ionic calcium by chelation. They also prevent any change in the shape or volume of rbc better than oxalate mixtures or potassium oxalate alone. The dry dipotassium salt of EDTA is preferred for sedimentation rates and packed cell volume since it does not change rbc volume. 0.2 ml of 2.5 per cent solution of this salt placed in a container and dried is enough for 5.0 ml of blood.

4. For ESR alone, 0.4 ml of 3.8 per cent sodium citrate solution mixed with 1.6 ml of blood is used. (Citrate solution is also used for storing blood collected from blood donors).

5. Anticoagulant and preservative combined. A mixture of 1.0 mg of thymol and 10 mg sodium fluoride will preserve blood for up to 5 days for non-protein nitrogen (NPN) and other biochemical determinations but it is always better to undertake the determinations as soon as time permits.

Venous or arterial blood can be collected under oil for determination of oxygen and carbon dioxide content, taking care that the blood sample is not exposed to air.

Note. Oxalates, EDTA and citrates are not used for treating patients requiring anticoagulant therapy. (Heparin, dicoumarol and others are used for this purpose).

B. Capillary Blood

The ball of the finger or the lobe of the ear are the usual sites for collecting capillary blood. In infants the heel or big toe can be used. It is essential to have all the apparatus ready

before giving a prick. If a lancet needle or a pricking gun is not available, a bayonet-pointed pricking needle should be used. Ordinary injection needles are useless for this purpose.

Clean the area with a cotton swab dipped in alcohol and allow the skin to dry completely. Apply a gentle pressure on the sides of the ball of the finger and prick the skin sharply and quickly to a depth of about 2 mm and release the pressure. The blood should start flowing freely and quickly if a good prick has been given. *Do not* press or squeeze the finger as the tissue fluid squeezed out along with the blood will dilute it. You may exert a slight tension outwards on either side of the prick with the thumbs in order to open the skin puncture more widely. If all efforts fail to obtain a sufficiently large drop of blood, a fresh prick on another finger should be given.

Wipe away the first two or three drops of blood with a dry cotton swab and fill the pipette from a fresh drop of blood. Blood films are also prepared from capillary blood for various purposes.

Precautions. (1) Examine the site selected for skin puncture. There should be no skin disease, inflammation or oedema. The finger should not be too cold or bloodless. (2) Do not squeeze the finger. The forearm or the hand may, however be squeezed or "milked" towards the fingers to facilitate blood flow. (3) All the apparatus should be ready before a finger prick is given. (4) The needle must be sterilized over a gas flame before using it on another person. (5) The first one or two drops should be discarded as these may contain epithelial or endothelial cells which may appear as artefacts on a blood film. The first drop is also diluted with tissue fluid, and false cell counts may result.

THE DILUTING PIPETTE

It is employed for the enumeration of red cells, white blood cells, platelets and eosinophils, by taking a volume of blood and diluting it to a known volume. The pipette should be clean and dry and the bead should roll freely in the bulb. A long rubber tube should be attached to the pipette with a plastic mouthpiece which can be held between the teeth. This permits the pipette to be held in a horizontal position and enables one to watch the blood entering the pipette.

1. Wipe away the first two or three drops of blood from the skin puncture with a dry cotton swab.
2. Hold the pipette horizontally and dip its end into a freshly-formed, good-sized drop of blood. Suck gently on the mouthpiece and draw blood to the required mark on the stem of the pipette. If a greater amount of blood has been drawn into the pipette, tap it gently against a finger nail or wipe the tip on your finger or on a piece of filter paper (keeping the pipette horizontal) in order to bring the blood to the desired mark. *Do not* try to blow out the extra blood.
3. Suck in the appropriate diluent placed in a watch glass (drawing the diluent from the stock bottle is likely to contaminate the solution with cells). The blood enters the bulb first, followed by the diluent; bring the level of diluted blood to 11 (WBC pipette) or 101 (RBC pipette) as the case may be.
4. Mix the contents of the bulb thoroughly for 2-3 minutes by rotating the pipette with its tip pressing against the palm of left hand (this is to prevent leakage of diluted blood from the tip), the right thumb and index finger holding it above the bulb where the rubber tube is attached (the tube may be removed to facilitate mixing). The glass bead in the bulb helps in mixing the contents of the bulb. (Allowance is already made for the volume of the glass bead). Mixing may be done by holding and rotating the pipette between the palms of the hands but

there is some risk of leakage especially if it is not held horizontally. If a pipette shaker is available, it may be used to advantage. RBC and WBC pipettes are described in details in the relevant experiments.

5. Charge the counting chamber with the diluted blood as described later. Clean the pipette thoroughly after use.

Experiment No. : 5.4

HAEMOCYTOMETER OR COUNTING CHAMBER

The student is advised to familiarise himself thoroughly with the counting chamber before using it. The chamber and the coverslip must be absolutely grease free. Use the piece of flannel provided for this purpose.

It is a single, solid, heavy glass slide. There are three narrow parallel platforms on its middle third portion and which extend across it. The central platform or "floor piece" is $1/10$ mm lower than the other two and is divided into two parts by a short transverse gutter or trench (Fig. 5.2). Each platform is ruled with a counting grid for red and white blood cells. When the coverslip is placed in position, a space of 0.1 mm deep is left between the under surface of the coverslip and the counting platform. Reticulocytes, platelets, eosinophils and bacteria can also be counted.

The counting chamber in common use has improved Neubauer ruling. There are 400 small squares grouped into 25 groups of 16 squares each by two lines called tram lines (three lines in some chambers). Squares for counting white blood corpuscles, four groups of 16 squares each, are also etched on

the counting platform as shown in Fig. 5.3. In every make, the dimensions and volumes of the squares are always specified on the chamber surface.

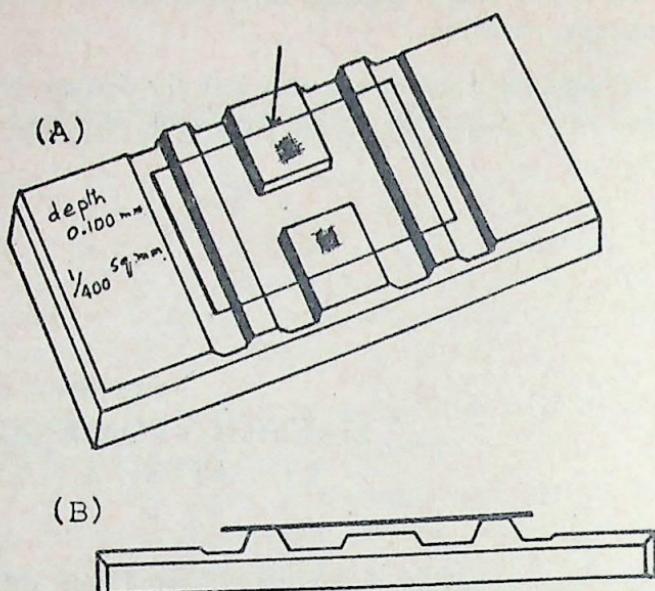


Fig. 5.2 Haemocytometer or counting chamber with improved Neubauer's ruling. (A) Surface view, with the cover slip in position. The locations of the counting grids on the two platforms ("floor pieces") are indicated. The arrow indicates the place where the tip of the pipette should be placed for "charging" the chamber. (B) Side view with the coverslip in position. The space between the underside of the coverslip and the surface of the platform is 0.100 mm in depth. The depth and the area of the smallest square are etched on the surface of the chamber.

Examine the chamber under LP and HP objectives, and locate and identify each type of square. If the lines are faint, rub some powdered black lead from your pencil to bring out the lines clearly. Clean the surface of the platform with flannel to remove the excess carbon particles.

Charging the Counting Chamber

Examine the chamber under the low power objective, without the coverslip, in order to understand the ruling. Compare what you see with the diagram of the ruling grid.

- i. Place the chamber on a flat surface with a blank white paper under it. Put a heavy coverslip on the surface of the chamber and 'centre' it.

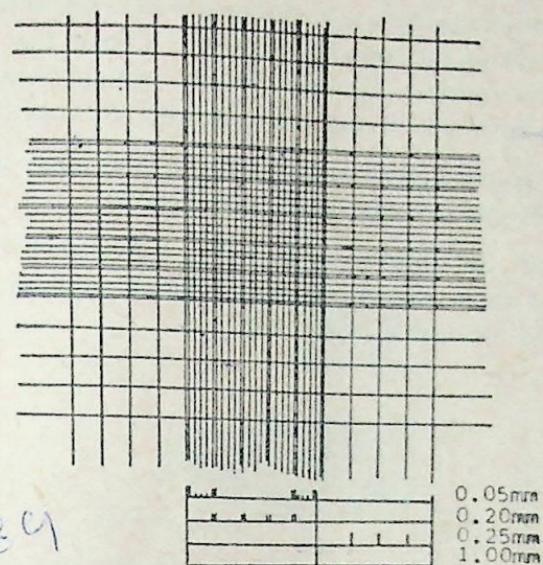


Fig. 5.3 Improved Neubauer's chamber. The four corner groups of 16 squares (side 0.25 mm) are used for counting WBC. The red blood cells are counted in five groups of 16 smallest squares each (side 0.05 mm). The grid also provides a convenient scale for measuring the size of small objects, like parasite eggs.

2. Mix the blood with the diluent thoroughly. Discard the first 2-3 drops of fluid from the pipette. Wipe the pipette and place its tip on the surface of the chamber touching the edge of the coverslip at an angle of 45° to the horizontal. Allow the diluted blood to flow evenly and slowly under the coverslip by capillary action. Remove the pipette quickly as soon as the counting platform is covered with the diluted blood. There should be no overflow into the central or side trenches as the excess fluid will lift up the coverslip and give false high counts. If any diluted blood spills over into the trenches, the chamber and the coverslip must be cleaned and the chamber recharged.

3. Allow the cells to settle for 2-3 minutes before putting the charged chamber on the fixed stage of the microscope.

4. Focus the lines and cells under low power before counting the particular cells in the appropriate squares. Move the fine adjustment screw continuously (i.e., rack the microscope up and down a little) to ensure that cells adhering to the underside of the coverslip are not missed, and also to allow for uneven settling of cells on the counting platform. Check that there are no large discrepancies between the cell counts from different parts (large differences necessitate recharging the chamber).

5. Get your notebook stamped by the laboratory technician and enter the counts in the appropriate squares.

6. Clean the pipettes with water, followed by acetone, sucked into them followed by air aspiration until these are dry and the bead rolls freely in the bulb.

Experiment No. : 5.5

PREPARATION OF A BLOOD SMEAR (BLOOD FILM)

The blood film will be needed for differential white cell counts, platelet and reticulocyte counts, and for the study of red cell morphology.

Method of spreading a blood film. 1. Get a skin puncture and discard the first two drops of blood. Hold a clean, grease-free glass slide along its long edges (*do not* touch the surface of the slide with your fingers) and touch one end of the slide surface to a newly-formed small drop of blood (or put a small drop of blood on the surface of the slide, about 1 cm from its end, directly from the needle of the syringe employed for collection of venous blood, before removing the needle from the syringe and expelling the rest of the blood into a container).

2. Put the slide flat on a table over a piece of white paper and support its end with the fingers of one hand. Grasp the sides of a second slide (the 'spreader') with the fingers and thumb of the other hand and hold it at an angle of 45° to the horizontal, as shown in the diagram (Fig. 5.4).

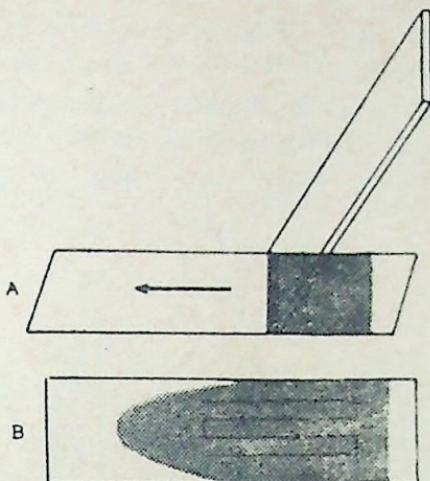


Fig. 5.4 (A) The method of spreading a blood smear. (B) The appearance of a well-prepared blood film, showing the movement of the oil objective over it.

3. Place the edge of the 'spreader' on the first slide just in front of the drop of blood and draw it back until it makes contact with blood. Allow the blood to spread along the junction of the two slides and wait until the blood almost reaches the edge of the slides. Steady the first slide and move the 'spreader' forwards with a single smooth motion (apply only a gentle pressure). The blood is actually pulled along the slide and not pushed. The smear should neither be very thin nor thick and should taper off into a tail. Dry the blood film by waving the slide in the air as soon as spread.

4. The dried smear should be buff coloured. Examine it under high power before staining to check that the spreading is satisfactory. The cells should lie separately and there should be no clumping of cells. Thick and uneven smears should be discarded. It is preferable to make 3-4 smears and stain them together. The best-stained film should be selected for microscopic examination.

Experiment No. : 5.6

ESTIMATION OF HAEMOGLOBIN (Hb) CONCENTRATION

Principle. Haemoglobin in the red blood corpuscles is converted into acid haematin. The brown colour so developed is matched against standard brown-tinted glass in the comparator by direct vision. Reading is taken directly as g Hb/100 ml blood. Capillary or venous blood may be used for this estimation.

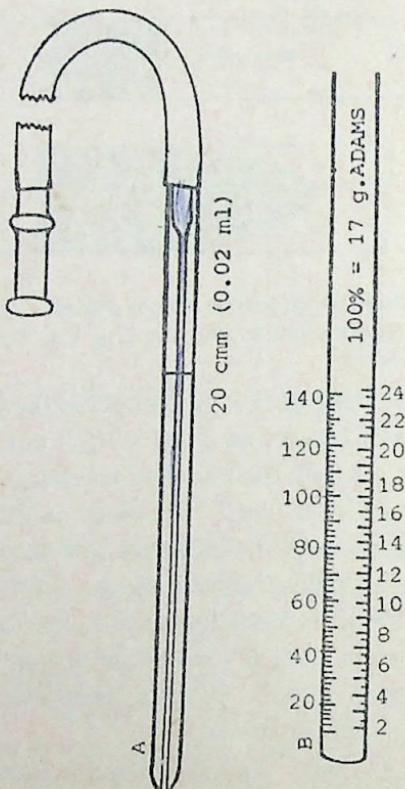


Fig. 5.5 (A) The haemoglobin pipette. It bears only one marking, indicating 20 cm m (0.02 ml). (B) Sahli—Adams haemoglobin tube. It bears graduations in g. per cent on one side, and in percentage on the other, 100 percent being equivalent to 17.3 g Hb. per 100 ml of blood. In other tubes the calibration is such that 100 per cent is equivalent to 14.8 g. The student should know how the tube is calibrated in these respects. Express your result in g per cent.

Apparatus and reagents. (A) Haemoglobinometer. (Sahli's). The box contains.

(i) *Comparator.* A slot accommodates the Sahli tube. Non-fading standard brown-tinted glass pieces are provided on either side of the slot for colour matching. An opaque white glass is fitted at the back to provide uniform illumination.

(ii) *Haemoglobin tube (Sahli Adams tube).* Graduated in g per cent of Hb (2-24) as well as percentage (20-140) (Fig. 5.5).

(iii) *Haemoglobin pipette.* It has only one mark, indicating 20 cmm (0.02 ml) blood. (see Fig. 5.5)

(iv) A thin glass-rod stirrer. Ordinary glass tube dropper with a teat.

(B) N/10 HCl. (0.1N-HCl) solution. Distilled water. Pricking needle. Cotton swabs. Alcohol.

Procedure. Read the instructions for collecting capillary blood (5.2) and follow the procedure strictly.

(1) Study the haemoglobin tube and note the graduations. Fill a clean and dry tube with N/10 HCl solution upto the mark 3 g (or 20 per cent) and put it aside. (2) Get a finger prick with a sterile needle and wipe away the first one or two drops of blood with a cotton swab. When a good-sized drop forms at the puncture site, dip the tip of the pipette in it and draw blood to the 20 cmm mark by gently sucking on the plastic mouthpiece fitted to the rubber tube. The tip should not press against the skin nor move out of the drop of blood, otherwise air will enter the pipette and the whole procedure will have to be repeated. (3) Wipe the tip of the pipette with cotton so that no blood is left sticking to its outside. Expel the blood into the Sahli tube containing the HCl solution. Suck a small amount of acid into the pipette and expel it again into the tube; repeat this twice and withdraw the pipette from the tube, making sure that no solution remains on or in the pipette. Mix the contents quickly but gently by shaking the tube before any clotting of blood occurs (avoid froth formation). Put the tube back in the comparator and wait for at least 10 minutes. During this time the red cells rupture, liberating the haemoglobin into the acid

solution ; the acid reacts with haemoglobin and converts it into *Acid haematin* which is brown in colour. (4) Take out the tube from the comparator, add a few drops of distilled water and stir the contents with the glass rod, gently, but well. Continue to add water drop by drop (or in larger amount according to experience), stirring the contents each time, till the colour of the solution matches the standard. (It is essential that the colour of the solution should be uniform throughout). Read the upper meniscus (coloured solution) and note the reading.

Results. The extent of error with this method is about 8-10 per cent, which can be reduced to about 5 per cent by taking three readings ; first, when the colour is slightly darker than the standard, second, when the colour exactly matches the standard and the third, when the colour is slightly lighter than the standard. The average of these three reading gives a more accurate result. Express your results as :

Haemoglobin concentration = ... g per 100 ml blood

Discussion. It is important to know the limitations of any particular method as haemoglobin may be present in the blood in many forms. Sahli's acid haematin method estimates oxyhaemoglobin and reduced haemoglobin ; carboxyhaemoglobin and methaemoglobin are not detected. The acid haematin is not in true solution, some degree of precipitation or turbidity may at times, therefore, interfere with colour matching. The method is otherwise quick and most convenient and is used as a routine in clinical work.

About 90 per cent of the dry weight of the red cells is made up of haemoglobin which plays a vital role in the normal functioning of the body. The entire amount of haemoglobin is carried inside the red cells, only a very minute amount (about 3 mg per cent) being present in the plasma. Were it present freely in the plasma, it would cause a tremendous increase in the osmotic pressure and would also be filtered out in the renal glomeruli and excreted in urine. Intravascular haemolysis due to any cause, releases large amounts of haemoglobin which is filtered out in the urine.

Normal levels of haemoglobin

	<i>Average</i>	<i>Range</i>
Males	= 15.8 g per cent	14-18 g per cent (16 ± 2)
Females	= 13.7 g per cent	12.0-15.5 g per cent (14 ± 2)

In the newborn, the haemoglobin level may be as high as 20 g per cent due to the high rbc counts. As the age advances, the level goes on decreasing and normal adult levels are reached by the age of 16-18 years. Weight and body surface area being equal in a male and a female, the Hb concentration is lower in the female. This is not due to menstruation solely ; the Hb level and the rbc counts are high in the males due to the action of the male hormone, testosterone.

Although the Hb content may vary considerably without serious consequences, any severe anaemia (low Hb content of blood) will cause tissue hypoxia. This estimation is, therefore, an invaluable diagnostic aid to the clinician.

Functions of haemoglobin. It serves two important functions—it carries oxygen and carbon dioxide, and acts as a buffer in maintaining the blood reaction. 1.0 g haemoglobin, when fully saturated with oxygen, carries 1.34 ml of this gas. Haemoglobin possesses a far greater affinity for carbon monoxide as compared to oxygen. The CO molecule attaches itself to the same site on the haemoglobin molecule where oxygen is normally attached, and in the process it displaces oxygen, thereby forming carboxyhaemoglobin (a better term is carbonmonoxyhaemoglobin). The great affinity of Hb for carbon monoxide is of importance in cases of poisoning with this gas, where pure oxygen, or even hyperbaric oxygen, besides artificial respiration, may have to be given to revive the patient. The haemoglobin molecule normally carries both O_2 and CO_2 simultaneously ; the combination of Hb and CO_2 is called carbaminohaemoglobin.

Types of haemoglobin. Haemoglobin is the red pigment present in the rbc and has a molecular weight of 64,450. (Hb has a characteristic absorption spectrum and hence a red colour). It is a globular molecule made up of 4 sub-units,

each of which contains a 'haeme' moiety conjugated to a polypeptide. Haeme is an iron-containing porphyrin called iron-protoporphyrin IX. The polypeptides are collectively referred to as the 'globin' moiety (portion) of the haemoglobin molecule. Hb appears in the red cells during the intermediate normoblast stage of development.

1. *Adult haemoglobin (Hb A).* The two types of polypeptides have alpha and beta chains. About 2 per cent of the haemoglobin in a normal adult is Hb. A₂ in which there is a difference in the aminoacid residues in the polypeptide chains.

2. *Foetal haemoglobin (Hb F).* The rbc of human foetus contain foetal haemoglobin (Hb F). The difference lies in the polypeptide chains. It disappears after birth but in some individuals it persists throughout life. At a given PO₂, Hb F carries more oxygen. This special property of Hb F is of great value to the foetus in utero.

3. *Abnormal haemoglobins.* Hb S, E, C etc. have been described. They differ in the sequence of aminoacids in the polypeptide chains and are acquired genetically. These abnormal haemoglobins may cause sickle-cell anaemia (Hb S), methaemoglobinemia, congenital erythrocytosis and other haematological disorders.

Other methods for estimating haemoglobin. 1. *Alkaline haematin method (Wu's method).* The principle is to convert haemoglobin into alkaline haematin which is in 'true' solution. All haemoglobin compounds are estimated, including methaemoglobin and sulphohaemoglobin.

2. *Oxyhaemoglobin method.* Blood is treated with sodium carbonate or ammonium hydroxide and the resulting oxyhaemoglobin solution is compared with standard grey screen in a photoelectric colorimeter.

3. *Haldane's Carboxyhaemoglobin method.*

4. *Cyanmethaemoglobin method.* Haemoglobin (reduced and oxygenated), carboxyhaemoglobin, and methaemoglobin i.e., all of the different compounds of haemoglobin found in normal blood, are rapidly converted into a single compound

which is compared with a standard solution in a photometer. This is achieved by mixing blood with a solution containing potassium cyanide and potassium ferricyanide.

5. *Tallquist method.* A drop of blood taken on a filter paper is compared with an arbitrary scale of red coloured round spots on a printed chart. This method has a number of errors which may be as high as 20-30 per cent.

6. Estimation of iron concentration, or oxygen carrying capacity of a given sample of blood.

7. Copper-sulphate specific gravity method is a rapid method for estimating the approximate concentration of haemoglobin in large surveys. This method was used extensively during World War II where an indication for the necessity of blood transfusion was immediately required.

Precautions. 1. There should be no blood sticking to the tip of the pipette, as this extra amount of blood will give a false high reading.

2. Do not take a larger volume of N/10 HCl than indicated because in cases of severe anaemia, the final colour produced will be lighter than the standard, and there is no way to concentrate the colour.

3. The colour of the standard tinted-glass should be checked occasionally against standard acid haematin solution.

4. Do not squeeze the finger, as the tissue fluid squeezed along with the blood will dilute it and give a false low result.

5. You must wait for at least 10 minutes for complete conversion of Hb into acid haematin.

6. Take the readings by holding the haemoglobinometer at full arm length, against good light. The tube should be placed such that the graduations on it do not lie directly in front and interfere in the matching of colour.

Questions (1) What difference would it make if the N/10 HCl is taken above the 20% mark? (2) Name the brown-

coloured compound formed by the action of HCl on haemoglobin. (3) Can you use N/10 HCl solution for diluting the mixture for matching the colour? Can tap water be used? (4) How can you reduce the 'personal' error while taking the reading? (5) What errors will give a false high or false low readings? (6) Why is it important to express your result in g. per cent rather than in per cent as such? (7) At what stage does haemoglobin appear in the red cells? (8) What is the fate of haemoglobin in the body? (9) What are the normal values of haemoglobin in children and in adults? Why is the value low in females? (10) How much oxygen is carried by 1g of haemoglobin? (11) What is anaemia? Name some common causes of anaemia.

Experiment No. : 5.7

THE RED CELL COUNT (ENUMERATION OF RED BLOOD CORPUSCLES)

Principle. The number of red cells is very high. The blood is, therefore, diluted 200 times, with an appropriate diluting fluid before the cells are counted in a haemocytometer. Their number in undiluted blood can then be calculated.

Apparatus and Reagents

1. Haemocytometer (counting chamber).
2. RBC pipette
3. Hayem's fluid
4. Pricking needle. Alcohol. Cotton. Thick coverslip.
5. Microscope with 10 × eyepiece. Coverslips.

Composition of Hayem's Fluid

Sodium chloride	: 0.5 g
Sodium sulphate	: 2.50 g
Mercuric chloride	: 0.25 g
Distilled water	: 100 ml

The mercuric chloride is antifungal and antibacterial. The diluting fluid is isotonic with blood.

The student is advised to familiarise himself thoroughly with the conuting chamber and the RBC pipette before starting the cell count. Consult 5.4. for the description of haemocytometer and the counting grid.

RBC Pipette. It has a narrow glass stem, graduated in tenths, with three markings—0.5, 1.0 and 101. The glass capillary tube widens into a small bulb containing a red glass bead. The bulb narrows again into glass capillary tube and at this point it is marked 101 (Fig. 5.6). Beyond this, a thin rubber tube with a plastic mouthpiece is attached. The red bead helps in mixing the contents of the bulb and for a quick identification of the RBC pipette.

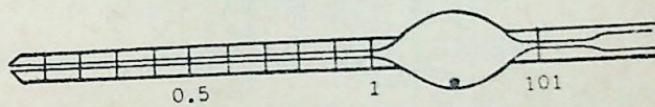


Fig. 5.6 The RBC pipette. It has three markings—0.5, 1 and 101.

The markings—0.5, 1.0 and 101, indicate the *volumes* of blood and diluent i.e., half volume, one volume and one hundred and one volumes (these markings *do not indicate c mm* of blood and diluent, a mistake often make by the students) Other markings, besides these three, permit smaller or greater dilutions to be obtained, e.g., in anaemia and polycythaemia.

Procedure. (1) Place the counting chamber with its 'centred' coverslip on the table near the microscope. Get a finger prick under aseptic conditions for obtaining capillary blood. Discard the first 2-3 drops and allow a good-sized drop to form. Draw

blood to the mark 0.5 in the RBC pipette, followed by Hayem's fluid to the mark 101. Mix the contents of the bulb thoroughly for one minute, taking care not to centrifuge the red cells towards the ends of the pipette (2) Discard the first 3-4 drops from the pipette and charge the counting chamber with the diluted blood as described before. Check that the coverslip has not been lifted up by the introduced fluid. Place the charged haemocytometer on the stage of the microscope and locate the central 1 mm sq area for red cell counting, under *low power*. Allow the cells to settle for one minute before starting the cell count. (3) Focus the lines and cells under *low power* to get a general impression of the distribution of the rbc over the counting grid (Fig. 5.7). If large discrepancies are

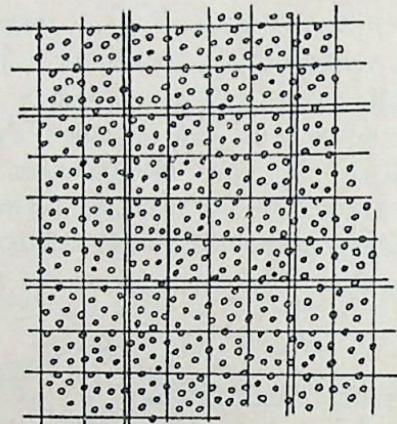


Fig. 5.7 Microscopic view of a 'charged' counting chamber. A group of 16 smallest squares (side— $1/20$ mm) is shown in the middle. Count the cells lying on the left and lower lines of a square and ignore those lying on its right and upper lines—these will be counted in the adjacent squares.

seen in different parts, then the spread is evidently uneven and the counts would be unreliable. Under such conditions, clean the chamber and the coverslip, and recharge. (4) The cells are to be counted in the four corner and one middle square—each with 16 smallest squares i.e., in 80 squares (Fig. 5.3). Switch to *high power* objective and adjust the position of the haemocytometer so that a group of 16 smallest squares comes

into view. Count the rbc in each square and those lying on its left and lower lines ; ignore those on its right and upper lines. In this manner one avoids counting a cell twice. A rubber stamp is available in the laboratory, which you may stamp into your note book. Enter the count for each small square as it is made. Check Fig. 5.3 again to ensure that you are counting the cells in the right squares.

Note. Continuously rack the microscope up and down a little, so that cells adhering to the undersurface of the coverslip are not missed.

Dilution obtained. The volume of the bulb is 100 (101—1=100). From the tip of the pipette to the mark 1.0, the stem contains Hayem's fluid only and which does not take part in the dilution. Thus 100 volumes of diluted blood (in the bulb) contain 0.5 volume of blood and 99.5 volumes of Hayem's fluid, giving a dilution of half in hundred. The dilution obtained, therefore, is one in two hundred i.e., 1:200 (200 times).

Calculation. The area of the smallest square is $1/400$ sq mm and volume $1/4000$ c mm. (side= $1/20$ mm, depth= $1/10$ mm, volume= $1/20 \times 1/20 \times 1/10 = 1/4000$ c mm (mm^3))

Example. Number of cells in 80 squares=480

$$\text{Number of cells in one square}=480/80=6$$

$$\text{Volume of one square}=1/4000 \text{ c mm } (\text{mm}^3)$$

Thus, there are 6 rbc in 1/4000 c mm of diluted blood.

The number of cells in 1 c mm will be $4000 \times 6 = 24000$. As the dilution employed is 1 : 200, the number of cells in 1 c mm of undiluted blood is $= 24,000 \times 200 = 4800000$. Express your result as—

$$\text{RBC count}=4.8 \text{ millions/c mm. } (\text{mm}^3)$$

Note. Multiplying the number of cells in 80 squares (480 in this example) by 10,000 will give the rbc count in millions per c mm blood i.e., by putting four zeroes in front of 480.

Normal RBC counts. In the newborn the count may be as high as 6-7.5 millions/c mm, 2—3 per cent of these being reti-

culocytes. The cells are larger ($MCV=100 \text{ cu}$; diameter= $8\mu\text{m}$), and the Hb concentration is high (15-25 g per cent). Normal adult levels are reached in 2-3 months.

	<i>Mean</i>	<i>Range</i>
Males (16-17 years)	= 5.0 millions/ c mm. (mm^3)	4.75-5.5 millions/ c mm (mm^3)
Females (16-70 years)	= 4.5 millions/ c mm. (mm^3)	4.0-5.1 millions/ c mm (mm^3)

The higher count in males is due to testosterone which stimulates erythropoiesis. With this method of rbc counting, the error is said to be ± 15 per cent. Thus, with a cell count of 5.5 millions/ c mm, the technical error of a single count comes to about ± 0.825 millions. RBC counts that are regularly below 4.5 millions/c mm in males and 4.0 millions/c mm in females should arouse suspicion of some disease, and a thorough check-up of the individual is indicated.

Electronic cell counters have been introduced in the recent years and these have been found to be quite accurate and efficient.

Discussion. The red cells are round, biconcave, elastic discs, thinner in the centre and without a nucleus ; the average diameter being $7.2 \mu\text{m}$ (normal range $6.9 \mu\text{m}-8 \mu\text{m}$). If a nucleus were present, it would not only use oxygen for its own metabolic activity, but also decrease the amount of haemoglobin that could be carried in the cell.

Site of Formation of RBC : In the foetus, erythropoiesis (formation of red cells) occurs in the bone marrow, spleen, liver, and thymus. After birth, it is confined to the red marrow only (ends of long bones, sternum, vertebrae, bones of the skull.) The red marrow contains pluripotent stem cells which can produce cells of any type. The pluripotent stem cell can self-replicate and differentiate to committed precursors—ie, cells that can give rise to only a specific cell line. The committed red cell precursor (proerythroblast) undergoes several divisions which pass through various stages of development (proerythroblast,

erythroblast, normoblast A, B and C, reticulocyte and erythrocytes). The daughter cells become progressively smaller, the cytoplasm changes colour (as seen in stained blood films) from blue to pink (haemoglobin appears in the normoblast B stage) the nucleus becomes smaller and is finally extruded. The resulting non-nucleated cell is called a reticulocyte since it contains RNA which appears as a network in the cytoplasm when stained appropriately. The reticulocytes mature into erythrocytes within a short time (the peripheral blood contains about 50,000 reticulocytes per mm³ i.e. 0.5 per cent). The total red cells in the body have been estimated at 25 million million/(25 × 10¹²), the normal rate of production being 15—20 ml of packed red cells per day.

Erythropoietin acts as the major established physiologic regulator of erythropoiesis. It is continuously produced by the glomerular or juxtaglomerular cells of the kidney (these cells sense tissue hypoxia) or it may be formed in the plasma by the action of a renal factor (renal erythropoietic factor, REF) that acts on a plasma globulin. Production of erythropoietin is increased by hypoxia which may result from a decreased blood haemoglobin, lung diseases and high altitude.

Erythropoiesis also requires adequate amounts of first class proteins, trace metals like iron, zinc, cobalt, copper; certain vitamins, especially C, B₁₂, folic acid and pyridoxine; and hormones, especially thyroxine, cortisol and androgens.

Life span of RBC. The average life span of a red cell is about 127 days. During this time the RBCs circuit the system some 300,000 times. The mature red cell, though without a nucleus, is not a dead cell in the usual sense. Since there is no nucleus, all the necessary enzymes needed to maintain its life span are present in it when it enters the circulation. Conversion of glucose to lactate by anaerobic glycolysis provides most of the energy to maintain the electrolyte and fluid balance inside the red cells; no energy being available from Kreb's tricarboxylic acid cycle (as there are no mitochondria). The fluid and electrolyte content is controlled by the sodium-potassium pump in the cell membrane.

After the reticulocyte stage, there is no way to determine the age of a particular cell. Some cells will be only a few days old while others would be quite old and ripe enough (from constant wear and tear) for removal from the circulation by the reticuloendothelial system (RES) cells. Within the RES cells, haemoglobin catabolism takes place, the porphyrins being converted to bile pigments, (which are subsequently excreted) and iron and amino acids extracted for reutilization by the body in the formation of new rbc.

Anaemia. The most accurate definition is a reduction in the circulating red cell mass. Since this information cannot be easily obtained, it is more practical to define anaemia in terms of Hb concentration and red cell counts. Different standards are adopted in different institutions. If the Hb is less than 10 g per cent or the rbc count less than 3.8 millions/c mm, a complete haemogram should be undertaken to find out the type and cause of anaemia.

Causes of anaemia. These are listed below in a simple classification.

(I) Loss of blood.

(II) Decreased formation of red cells and haemoglobin.

1. Iron deficiency (Microcytic hypochromic anaemia).
2. Deficiency of vitamin B₁₂ or Folic acid (Pernicious anaemia ; macrocytic normochromic).
3. Malnutrition—especially protein deficiency.
4. Bone marrow failure (Aplastic or hypoplastic anaemias).

(III) Increased destruction of red cells (Haemolytic anaemias).

1. Defects in the red blood corpuscles. Abnormal shapes due to enzyme deficiencies. Congenital.
2. Incompatible transfusions and haemolytic disease of the newborn.
3. Drugs and other agents which cause intravascular haemolysis.

IV. Anaemias associated with other diseases.

1. Chronic diseases : Tuberculosis, infections, malignancies.
2. Anaemias due to endocrine disorders—e.g., myxoedema.
3. Anaemias due to liver, spleen, and kidney diseases.

Polycythaemia. The term signifies an increase in the number of circulating rbc above the normal value. Differentiation must be made between absolute polycythaemia (total red cell mass increased) and relative polycythaemia. Polycythaemia is seen in the following conditions.

1. Polycythaemia of the newborn.
2. Polycythaemia resulting from hypoxia due to any cause—lung disease, high altitude, congenital heart disease etc.
3. Polycythaemia vera is of unknown etiology (erythremia).
4. Polycythaemia resulting from haemoconcentration—loss of fluid in acute vomiting and diarrhea, abnormally low fluid intake with profuse sweating ; and loss of plasma from body burns.

Erythrocytosis is a better term to describe absolute polycythaemia, where there is an increase in the total red cell mass which occurs in response to some known stimulus.

Precautions. 1. The Hayem's solution should be drawn into the pipette soon after blood has been drawn to the 0.5 mark. Every effort should be made to prevent clotting of blood in the pipette.

2. There should be no spilling of diluted blood from the pipette while mixing the contents of the bulb.

3. If any diluted blood spills over into the trenches, the chamber must be cleaned and recharged as the diluted blood in the trench will lift the coverslip, giving a false high count. The chamber and the coverslip should be washed with water and then alcohol. Dry both the chamber and the coverslip very carefully with a piece of cloth which does not leave any cotton threads. A repeatedly washed and clean cotton handkerchief serves the purpose well.

4. Dilution of the blood with tissue fluid should be prevented.

Questions : (1) What are the dimensions of the smallest square of the Neubauer's chamber ? (2) What is the composition of Hayem's solution ? Name the function of each component. (3) What do the markings on the RBC pipette signify ? (4) When blood is taken upto the mark 0.5 and diluted upto 101, why is the dilution obtained 200 times and not 202 times ? What is meant exactly by 200 times dilution—how much blood and how much Hayem's solution ? (4) Why is a thick coverslip preferred for the counting chamber ? (5) What is the life span of red cells ? How is it estimated ? (6) Describe the stages of erythropoiesis. What factors are required for normal erythropoiesis ? What is the physiological stimulus for normal red cell formation ? (7) What is anaemia ? How would you classify it ? Name the common causes of anaemia in this part of the country. (8) What is polycythaemia and what are its causes ? How does it affect circulation ? (9) What is the primary defect in pernicious anaemia and in microcytic hypochromic anaemia ?

Experiment No. : 5.8

DETERMINATION OF PACKED CELL VOLUME (PCV)* SYN : ESTIMATION OF HAEMATOCRIT (Hct)

Principle. A sample of blood to which an anticoagulant has been added is centrifuged in a haematocrit tube. The rbc are packed towards the bottom of the tube by the centrifugal force, and reading of the packed cells, in percentage, is taken.

Apparatus. 1. *Wintrobe tube.* (Fig. 5.9.) (Length—11 cm). It is open at the top but closed at its lower end and is graduated

*Group demonstration.

from 0-10 cm (100 mm) from above downwards on one side (for ESR) and 10-0 cm on the other side (for PCV).. It's bore diameter is 2.5 mm.

2. Centrifuge machine.

Pasteur pipette. It is an ordinary glass tubing drawn to a long thin nozzle about 13 cm in length. Rubber teat.

3. Sample of venous blood to which an anticoagulant has been added.

Procedure. (1) Using a Pasteur pipette, fill the Wintrobe tube with blood, starting at its bottom and withdrawing the pipette as the tube is filled from below upwards. Bring the blood column to the 'O' mark. Remove air bubbles, if any, from the top of the column of blood so that it stands exactly at 'O'. (2) Centrifuge the tube for about 20 minutes at 2500 rpm. Take the reading of the packed cells, centrifuge the tube again for 5 minutes and note the reading. Repeat the procedure if there is a difference. Final reading is recorded when three consecutive readings are identical i.e., when the red cells have been fully packed.

Observations and results. The upper level of packed red cell is quite sharply and distinctly visible. After centrifugation, the blood is separated into three layers ; a tall bottom layer of packed red cells, a very thin middle layer of wbc and platelets (the 'buffy layer') and the top layer of clear plasma. The percentage of the height of red cell volume constitutes the packed cell volume or haematocrit. For example, if the level of the red cell volume is at 40 mm the PCV is 40 per cent i.e., out of 100 volumes of whole blood, 40 volumes are red cells and 60 volumes plasma. (Do not confuse with the graduations meant for ESR).

Discussion. When whole blood is centrifuged in a tube, the red cells are packed together at the bottom of the tube by the centrifugal force as these are heavier than the liquid plasma. Packed cell volume represents the volume of cells per unit volume of whole blood and is expressed as a percentage. Even when the red cells are fully packed, about 2 per cent of plasma

remains trapped in between the cells, this percentage being more if the red cells are abnormal in shape (e.g. spherocytosis).

If it is not possible to obtain blood from a vein, a microhaematocrit method is employed. Graduated glass capillary tubes (heparinized) are filled with blood from a skin puncture and are placed in a carrier which is then fixed in a metal frame and rotated on a centrifuge. (Daland's method).

If the PCV is carried out in a special high-speed centrifuge (12,000 rpm for 3 minutes) with the microhaematocrit method, this test is one of the most accurate in clinical haematology. Duplicate samples run simultaneously agree within 1 per cent.

Changes in PCV affect the maximum transport rate of oxygen. A low haematocrit decreases the maximum content of oxygen in a volume of blood due to less haemoglobin, whereas a high haematocrit decreases the maximum cardiac output because of the increased viscosity. For a particular individual there is a haematocrit at which the rate of oxygen transport is optimal.

Normal values. The average value is 45 per cent, when the rbc are of normal size and volume and the count is 5.0 millions/c mm.

	<i>Average</i>	<i>Range</i>
Males :	47 per cent	38-54 per cent
Females :	42 per cent	36-47 per cent

The low Hct in females is due to the relatively low rbc count.

Variations in PCV. The haemetocrit may be increased or decreased under various physiological as well as pathological conditions.

(A) High PCV is seen in :

1. Polycythemia of the new born, (physiological).
2. Polycythemia in subjects living at high altitudes (Physiological).
3. Dehydration resulting from profuse sweating without adequate fluid intake, (physiological).
4. Dehydration resulting from severe vomiting and diarrhea or gastroenteritis. (pathological).

5. Burns. Extensive skin burns lead to exudation of plasma from the burnt areas, leading to haemoconcentration, (pathological).

(B) Low PCV is found in :

1. Anaemias of various types (pathological).
2. During pregnancy the value is low because of increased plasma volume and haemodilution (Physiological).
3. In females, the red cells count is low (Physiological).

Precautions. 1. The anticoagulant should not affect the size and shape of red cells.

Proper anticoagulant must be used to prevent coagulation of blood in the haematocrit tube.

3. Blood should be centrifuged for an adequate time and the final reading taken when three consecutive readings are identical.

Questions. (1) What does PCV signify ? What is haematocrit ? (2) Name the conditions in which PCV is raised or lowered ? (3) Why is the haematocrit value of venous blood slightly more than that of arterial blood ?

Experiment No. : 5.9

NORMAL BLOOD STANDARDS

A. Calculation of Absolute Corpuscular Values

In order to determine the condition of the erythrocytes, the following data are needed :

(1) The erythrocyte volume i.e. PCV. (2) The haemoglobin concentration. (3) The red cell count.

Much information can be obtained by determining the relationships between these values in diagnosing the cause of anaemia.

1. Mean corpuscular volume (MCV). This is the average or mean volume of a single red cell expressed in cubic microns ($\text{c}\mu$). There is no simple method for measuring the volume of a single red cell directly, however, the mean volume of a cell can be calculated from the PCV and the red cell count.

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count in millions/c mm}}$$

$$\text{or } \frac{\text{PCV per liter of blood}}{\text{RBC count in millions/c mm}}$$

Example. PCV = 45 per cent ; RBC count = 5.0 millions/
c mm (mm^3)

$$\text{MCV} = \frac{45 \times 10}{5.0} = 90 \text{ cubic microns} (\text{c}\mu, \mu^3)$$

How the above calculation is derived :

$$\begin{aligned} \text{PCV of cells in 100 ml blood} &= 45 \text{ ml} \\ &= 45 \times 10^{12} \text{ c}\mu \quad (1 \text{ ml} = 10^{12} \text{ cubic microns}) \end{aligned}$$

$$\text{Number of cells in 1 c mm blood} = 5.0 \times 10^6$$

$$\begin{aligned} \text{Number of cells in 100 ml blood} &= 5.0 \times 10^6 \times 10^5 \\ &\quad (100 \text{ ml have } 10^5 \text{ c mm}) \end{aligned}$$

Since the volume of 5.0×10^{11} cells in 100 ml blood is

$$= 45 \times 10^{12} \text{ c}\mu$$

$$\text{The volume of one cell is } = \frac{45 \times 10^{12}}{5.0 \times 10^{11}}$$

$$= \frac{45 \times 10}{5.0} = 90 \text{ c}\mu (\mu^3)$$

Normal range = 75—94 $\text{c}\mu$ (μ^3)

Mean corpuscular volume by itself is a reliable indicator of macrocytosis, except in spherocytosis, in which the diameter is relatively reduced, and the volume increased so that the relationship between the volume and diameter doesn't hold true.

2. Mean corpuscular haemoglobin (MCH). This is also determined indirectly. It expresses the average haemoglobin content of a single red cell in micromicrograms or picrograms. It can be calculated from the rbc count in one c mm blood and the haemoglobin concentration per cent (e.g., Hb per 100 ml blood).

$$\text{MCH} = \frac{\text{Hb in g per cent} \times 10}{\text{rbc count in millions/c mm}}$$

$$\text{or } \frac{\text{Haemoglobin per liter blood}}{\text{rbc count in millions/c mm}}$$

Example. Hb concentration per cent=15.0 g;
rbc count=5.0 millions/c mm

$$\text{MCH} = \frac{15 \times 10}{5.0} = 30 \mu\mu\text{g} = 30 \text{ pg. (picograms)}$$

How the above calculation is derived :

Haemoglobin content of cells in 100 ml blood=15.0 g per cent

$$= 15 \times 10^{12} \mu\mu\text{g.} \quad (1\text{g}=10^{12} \mu\mu\text{g})$$

$$\text{Number of rbc in 1 c mm blood}=5.0 \times 10^6$$

Number of rbc in 100 ml blood= $5.0 \times 10^6 \times 10^5$ (100 ml have 10^5 c mm). Since the Hb content of 5.0×10^{11} cells is

$$= 15 \times 10^{12} \mu\mu\text{g.}$$

$$\text{the Hb content of one red cell is} = \frac{15 \times 10^{12}}{5 \times 10^{11}}$$

$$= \frac{15 \times 10}{5} = 30 \mu\mu\text{g} \quad (30 \text{ pg.})$$

$$\text{Normal range}=27-32 \mu\mu\text{g.}$$

(The numerator is the g Hb per 100 ml blood, and the denominator—the rbc count in millions per cubic millimeter blood). MCH thus represents the weight of haemoglobin content in the average red blood cell).

3. Mean corpuscular haemoglobin concentration (MCHC). This value represent the relation between *haemoglobin saturation*

and *cell volume*. It does not take into account the number of rbc and is, therefore, better described by the simple term haemoglobin concentration. MCHC thus represents the saturation of rbc with haemoglobin. Beyond a certain saturation limit, the red cells cannot contain more haemoglobin. The value therefore, represents the actual haemoglobin concentration in the blood and is thus a true indicator of iron deficiency. However, certain anaemias with low MCHC will not respond to iron as it is apparently not utilized. MCHC is calculated as shown below :

$$\text{MCHC} = \frac{\text{Hb in g per 100 ml blood}}{\text{Volume of packed rbc/100 ml blood}} \times 100$$

Average=33 per cent

Normal range=32—38 per cent.

Example. Hb=15 g per cent ; PCV=45 per cent

$$\text{MCHC} = \frac{15}{45} \times 100 = 33.3 \text{ per cent.}$$

If MCHC is within normal limits, the rbc are normochromic, if it is below the normal range the rbc are hypochromic. There is probably no condition known where the MCHC is more than 38 per cent. In macrocytosis (cell volume more than normal—it may be 95 to 160 μ) seen in pernicious anaemia, the *absolute* amount of Hb present in the red cells may be as high as 45—50 pg (upper normal limit=32 pg), but the *percentage* haemoglobin concentration will be normal (35 per cent).

It is commonly seen that in iron-deficiency type of anaemia, the red cells are microcytic hypochromic, whereas in pernicious anaemia, the rbc are macrocytic normochromic. If, however, there is associated iron deficiency in pernicious anaemia, the rbc are macrocytic hypochromic. Wintrobe (1933) examined the blood of different mammals, reptiles, and fish and found a relatively constant MCHC. The MCH however, varied greatly and in general, was inversely proportional to the rbc count.

4. **Mean corpuscular diameter (MCD).** This is determined by direct micrometric measurements of red cells in a stained film. The range is from 6.9 μm to 8.0 μm , with an average of

7.2 microns. MCD is an important factor for calculation of mean corpuscular average thickness. Any error is greatly magnified because the figure has to be squared. An experienced haematologist can get a reasonable impression of any change in the diameters of rbc in a blood film.

Price-Jones curve represents the distribution of the size of red blood cells. The curve is prepared—with diameter of rbc in microns and percentage of cells. A shift of the curve to the left is seen in macrocytosis.

5. Mean corpuscular average thickness (MCAT). The normal red cell is a biconcave disc and its thickness varies at different points, therefore, the average thickness concerns the cell as a whole. There is no technique available for measuring the average thickness directly, it has to be calculated by assuming that the red cell has the form of a cylinder of which the height represents the average thickness of the cell. The mean thickness or height is arrived at by balancing the concavities against the convexities so as to obtain an average. Thus having made the assumption of a cylinder, the ordinary formula can be applied :

$$\text{Volume} = \text{area} \times \text{height i.e., } \mu r^2 \times h$$

$$\text{Therefore, height} = \frac{\text{Volume}}{\mu r^3}$$

B. Calculation of Haematological Indices (rbc Indices)

The results obtained in a particular case are compared with the normal values. These indices have now been almost abandoned in favour of absolute values. Nevertheless, occasional use of these indices is made in clinical haematology.

The three traditional blood indices, colour, volume and saturation are relative measures of the haemoglobin, size and content of red cells.

1. Colour index (CI). If the haemoglobin and red cell count are expressed in the same units a relation can be established between them. This relation is constant in a normal person. The unit adopted is the 'percentage of normal'; it being already

assumed that a normal person has 100 per cent Hb and 100 per cent red blood corpuscles. Colour index is the ratio of percentage of Hb compared with a normal 100 per cent to the percentage of the number of rbc also compared with a normal 100 per cent.

$$\text{Colour index} = \frac{\text{Haemoglobin (percentage of normal)}}{\text{Red cells (percentage of normal)}} \\ = \frac{100}{100} = 1.0$$

Traditionally, and for convenience, the *normal 100 percent* of red cells has been fixed at 5.0 millions/c mm, irrespective of age and sex. The *normal 100 per cent* for haemoglobin is 15.0 g/100 ml blood (14.8 g per cent is taken as the *normal 100 per cent* by some authorities).

Calculation of colour index. For this we require the Hb concentration and the rbc count determined in an individual.

For example—Hb concentration=16.0 g per cent
rbc count=6.0 millions/c mm

$$\text{CI} = \frac{\text{Hb per cent of normal}}{\text{rbc count per cent of normal}} = \frac{\text{g per cent of Hb found}}{\frac{\text{Normal Hb}}{\text{rbc count found}}} \\ \frac{\text{Normal rbc count}}{\text{}}$$

(1) 5.0 million rbc/c mm=100 per cent

6.0 million rbc/c mm will be $\frac{6}{5} \times 100 = 120$ per cent

(2) 15.0 g Hb=100 per cent

16.0 Hb will be $\frac{16}{15} \times 100 = 107$ per cent

The colour index in this case= $\frac{107}{120} = 0.89$

Normal range of colour index=0.85—1.15.

Normal colour index and its limitations. The MCH and the colour index (CI), both serve to indicate the average amount of haemoglobin per red cell. A change in the CI means that the

average Hb content of the average red cell is increased or decreased. A normal colour index, if not unity, is usually less than 1.0 rather than greater than 1.0, but it is the definite range that is important. Both MCH and CI are higher than normal in most of the macrocytic anaemias (pernicious anaemia, anaemia of sprue and others); they are less than normal in most of the secondary anemias. It is essential to remember that *the Hb and the rbc count may decrease simultaneously in such a way that the colour index is within normal range or even 1.0.* Thus the colour index by itself is not of much clinical significance, because it is only a crude indication of cell size (as it depends partly on the cell size and partly on Hb concentration). Perhaps the only utility of colour index lies in orienting a type of anaemia and in that the amount of alteration may give some indication about the average red cell size. It is quite obvious, therefore, that various combinations of cell size and saturation may occur which give a CI that may be wrongly interpreted.

2. **Volume index (VI),** where PCV of 42 per cent is 100 per cent.
3. **Saturation index (SI),** where 35 per cent MCHC is taken as 100 per cent.
4. **Thickness index (TI),** where 2.1μ is considered 100 per cent; are calculated in the same manner as *colour index.*

Significance of the absolute values and indices. The advantage of the absolute values as compared to the index system is that the results are calculated on the observed figures without reference to any arbitrarily fixed normal values. The normal values have quite a wide range and the absolute values very much emphasize this range. In the index system, however, the normal figure of 1.0 is likely to be considered as an authoritatively asserted normal and the *important aspect of range of normality* may be forgotten.

Questions : (1) How is colour index calculated? What is its significance? (2) Name the conditions in which MCH can be higher than normal? (3) Is there any condition in which MCHC can be higher than 38 percent? If not, what is the reason? (4) What is the advantage of the absolute values as compared to the index system?

Experiment No : 5.10

TOTAL LEUCOCYTE COUNT (TLC)

Principle. The blood is diluted with a suitable diluting fluid which destroys the red blood corpuscles and stains the nuclei of the white blood cells. The leucocytes are then counted in a haemocytometer and their number in undiluted blood calculated. (The leucocytes actually look not white but colourless).

Apparatus and Reagents

1. Haemocytometer (counting chamber)
2. WBC pipette
3. Turk's fluid

Composition of Turk's fluid

Glacial acetic acid	...1.5 ml
Gentian violet	
1 per cent solution in water)	...1.0 ml
Distilled water to 100 ml.	

WBC pipette. It has a ground conical tip and a fine-bored narrow glass stem graduated in tenths (as in RBC pipette) and bears three markings—0.5, 1.0 and 11. The glass capillary tube widens into a bulb which contains a white bead. The bulb narrows again into a glass capillary tube and at this point, it is marked 11 (Fig. 5.8.) Beyond this, a thin rubber tube with a plastic mouthpiece is attached. The white bead helps in mixing the contents of the bulb and for a quick identification of the WBC pipette.

The markings 0.5, 1.0 and 11 indicate *volumes* and not cubic milli metres of blood and diluent.. Other graduations permit smaller or greater dilutions, e.g., in leucopenia and leukaemias. If blood is drawn upto the mark 1.0 then the dilution will be 1 in 10.

Procedure. (1) Place a counting chamber with its 'centred' coverslip near the microscope. Get a fingerprick under aseptic conditions and draw blood to the mark 0.5, followed by Turk's

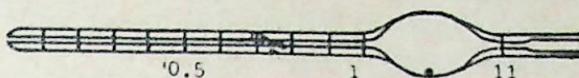


Fig. 5.8 : The WBC pipette. It has three markings—0.5, 1 and 11

fluid to the mark 11. Mix the contents of the bulb for 2 minutes. (2) Discard the first 2-3 drops of the diluted blood and charge the counting chamber, taking the necessary precautions. Place the 'charged' haemocytometer on the stage of the microscope and allow the cells to settle for two minutes. (3) Focus the lines and the cells to get a general impression of the distribution of cells. Switch to high power and count the cells in the four groups of 16 squares each. The procedure of counting the cells is the same as that for red cell counting. Rack the microscope continuously up and down with the fine adjustment screw, so that the cells adhering to the undersurface of the coverslip are not missed. Enter the count for each square as it is made, in your note-book.

It is difficult for a beginner to differentiate white cells from dust particles which is the cause of confusion and results in unreliable counts. The student is, therefore, advised to identify and count the cells *under high power* in the beginning. After some practice has been gained the cells may be counted under low power and reduced illumination, as the group of 16 squares (in mm square) is visible in one field.

The white cells (these are 'white' in comparison to red cells which contain haemoglobin) appear as round, faintly dark and refractile dots under low power, whereas under high power the stained nucleus is visible with clear granular cytoplasm around it (even the lobing of the nucleus of granulocytes can be made out.) The size and shape (often angular) of the dust particles varies and their colour is yellowish or brownish.

Dilution obtained. The volume of the bulb is 10 ($11-1=10$). From the tip of the pipette to the mark 1.0, the stem contains only Turk's fluid which does not take part in the dilution of blood. Thus 10 volumes of the diluted blood (in the bulb) contain 0.5 volume of blood and 9.5 volumes of Turk's fluid, giving a dilution of half in ten or one in twenty i.e, 1 : 20. The blood is thus diluted 20 times.

Calculations. The volume of one small square is $1/160 \text{ c mm}$ (side = $1/4 \text{ mm}$, area = $1/4 \times 1/4 = 1/16 \text{ sq mm}$, depth = $1/10 \text{ mm}$, volume = $1/4 \times 1/4 \times 1/10 = 1/160 \text{ cubic millimetres}$).

Example. Number of cells in 64 squares = 192

$$\text{Number of cells in one square} = 192/64 = 3$$

$$\text{Volume of one square} = 1/160 \text{ c mm} (\text{mm}^3)$$

Thus, there are 3 cells in $1/160 \text{ c mm}$ of diluted blood.

The number of cells in 1 c mm blood will be
 $160 \times 3 = 480$

As the dilution employed is 1 : 20, the number of cells in 1 c mm of undiluted blood is $= 480 \times 20 = 9600/\text{c mm}$ of blood.

Express your result as : TLC = $9600/\text{c mm} (\text{mm}^3)$.

Discussion. The normal total white cell count ranges between 4,000 and 11,000/c mm blood. As the red cell counts range between 4.5 to 5.5 millions/c mm, it is impossible to count the white cells unless the red cells are first destroyed. The acetic acid in the Turk's fluid haemolyses the red cells *without affecting the white cells*, and gentian violet (methyl violet can be used) stains the nuclei of the leucocytes which makes their identification easy.

The leucocytes may be considered as part of a broader system that provides protection against infections—namely the reticuloendothelial system (RES). The RES includes all phagocytes, mobile and fixed, which are mainly situated in the liver, spleen, lymph glands, lungs, gastrointestinal tract, and bone marrow. The leucocytes constitute the mobile defences of the body. They can pass through the vascular endothelium and enter the tissue spaces by a process called diapedesis. They are

obviously attracted by chemical substances (chemotaxis) released by bacteria and can engulf (phagocytosis) and digest foreign substances.

Two major groups of leucocytes are recognised—the *granulocytes* (polynuclear) and *agranulocytes* (mononuclear), depending on the presence of granules in the cytoplasm and the type of nucleus. The granulocytes include neutrophils, basophils and eosinophils according to the staining characteristics of their granules. The agranulocytes include lymphocytes and monocytes. All granulocytes contain the enzyme myeloperoxidase and the peroxidase reaction helps to differentiate these cells (myeloid series) from those of the lymphoid series.

The life span of granulocytes is about 5-7 days, their entire population turning over about two and a half times each day. Thus, over 100 billion cells are produced by the bone marrow each day. Lymphocytes survive for about 100 days while monocytes remain in the circulation for a few hours only.

The number of circulating leucocytes is very precisely controlled. The nature of chemical substances which stimulate their formation and release is complex and includes nucleic acid or its salts, and substances released from the site of infection or from the nuclei of disintegrating cells. These substances have been collectively called *granulopoietins*. A splenic hormone has been suggested to regulate their delivery from the bone marrow, though spleen itself disposes off old leucocytes.

Leukaemias. In these diseases there is an abnormally large number of a specific type of leucocyte within the body, though there is no demonstrable cause. The etiology is unknown, but certain factors are believed to be important, for example, ionizing irradiation, chemical leukaemogens and genetic influences. Immature cells are poured into the circulation in large numbers and the TLC may be as high as 100,000 to 300,000/c mm blood, and may exceed one million/c mm in chronic myeloid leukaemia. Myeloid and lymphoid leukaemias are differentiated according to the type of leucocytes involved.

Variations in Total Leucocyte Count

Variations in TLC are encountered in some physiological and many pathological conditions. Increase in number beyond 11,000/c mm is called leucocytosis. (Unless specified otherwise, this indicates an increase in the number of circulating granulocytes) Leucopenia refers to the condition where the number decreases below 4000/c mm.

Leucocytosis. The total count increases under certain physiological conditions. About 95 per cent of individuals have the normal range of 4000-11000/c mm. As a rule no significance is attached to fluctuations of less than 50 per cent in TLC and 10 per cent in differential count.

A. Physiological leucocytosis is seen in :

(1) New born infants (15000-20000/c mm) and upto the age of one year. (2) Conditions associated with stress and strain physical exercise, mental stress, food intake and digestion (digestion leucocytosis) and exposure to sun and very low temperatures. In these conditions there is no formation of new white cells (no shift of 'Cooke-Arneth count' to left) but a mobilization of cells from the internal organs (spleen, for example). 3. Pregnancy (especially in primiparae) and parturition-possibly due to a combination of tissue injury, haemorrhage and severe exertion.

Physiological leucocytosis (which has no clinical significance) is unaccompanied by eosinopenia, a feature commonly seen in the leucocytosis produced by infections.

B. Pathological leucocytosis is encountered in :

(1) Infections with pyogenic bacteria (may be local or general)—boils, abscess, appendicitis, pneumonia, sepsis etc. The leucocytic response may be quite marked in infancy and childhood. (2) Haemorrhage—the maximum response occurs in 8-10 hours but normal count is restored in 3-4 days unless infection supervenes. (3) Burns—maximum response occurs in 5-15 hours and the count returns to normal in 2-3 days. (4) Malignant diseases—increased counts are seen in about half the patients ; secondary infection enhances leucocytosis.

Leucopenia. The term is used when the TLC is below the normal lower limit of 4000/c mm.

A. Physiological. A physiological fall in the count is unusual and rare. Exposure to cold, even under arctic conditions and in spite of acclimatisation, may reduce the count to only slightly below 4000/c mm.

B. Pathological. This is almost always due to neutropenia and is seen in :

Infections—(non pyogenic). (a) Most commonly in typhoid and paratyphoid fevers. (b) Less frequently in influenza, malaria, smallpox, mumps and in toxæmias resulting from overwhelming infections. (2) *Drugs*—chloramphenicol, used in the treatment of typhoid, is known to suppress the bone marrow and produce leukopenia. It may also be suppressed in susceptible individuals with some drugs in common use like the sulpha group, aspirin etc. Cytotoxic drugs used in the treatment of malignant disease and leukaemias commonly cause pancytopenia (decrease in the number of all types of blood cells) by their action on the bone marrow. (3) Repeated exposure to X-ray and radium. (4) Blood disease and diseases of bone marrow—aplastic anaemia, pernicious anaemia and aleukaemic stage of leukaemia. (5) Poisoning with arsenic, dinitrophenol, antimony and other chemicals depress the bone marrow. (6) Malnutrition, starvation, extreme weakness and debility.

It is important to remember that leukopenia resulting from any cause makes an individual more prone to pyogenic and other infections.

Questions : (1) When 0.5 volume blood is diluted to the mark 11, why is the dilution obtained 20 times and not 22 ? What is meant by 20 times dilution—how much blood and how many volumes of Turk's fluid ? (2) What is the composition of Turk's fluid ? What is the function of each constituent ? (3) What is the normal range of TLC ? (4) What is meant by leucocytosis ? Name the physiological and pathological conditions that may cause it. (5) What is leucopenia ? Name the common conditions that cause it. (6) Where are leucocytes formed ? What is their life span and role in the body ?

Experiment No. : 5.11

DIFFERENTIAL LEUCOCYTE COUNT (DLC) SYN : DIFFERENTIAL WHITE COUNT

Principle. A blood film stained with Leishman's stain, is examined under immersion oil and the different types of white blood cells are identified. The percentage distribution of these cells is then determined.

Apparatus and reagents. Microscope. Clean, grease-free standard glass slides. Leishman's stain in a drop-bottle. A drop-bottle and a wash-bottle of distilled water. Pasteur pipette.

Leishman's stain. It contains Leishman's powder (eosinate of methylene blue—made from eosin and methylene blue dyes) dissolved in acetone-free absolute methyl alcohol. The reason why the undiluted stain is first poured over the blood smear is that the alcohol fixes the smear so that the film will not be washed out by water when the stain is diluted later.

Preparation of the stain is a tedious and time-consuming process ; it is best bought in commercially available packages containing the powder and solvent along with the instructions for the preparation of the stain. This staining procedure is probably one of the simplest and most precise technique for staining blood for diagnostic purposes.

Procedure (1) Prepare a blood film according to the technique described in 5.5 and place it on the staining rack assembled over a sink (a staining dish may be used). Pour 8-10 drops of Leishman's stain over the slide to cover the blood smear and allow it to stand for 2 minutes. (2) Add an equal volume of distilled water from a drop-bottle and mix the water and stain by tilting the slide first one way and then other, or by gently blowing on different places on the slide through a Pasteur pipette. Allow to stand for 6 minutes. (3) Drain off the diluted stain in a stream of distilled water from a washbottle for about 20 seconds and allow the slide to remain on the staining rack for 1-2 minutes, with the last wash covering it. (4)

(4) Put the slide against a support in an inclined position, stained smear facing down (this is to prevent dust particles settling on the blood film) and allow it to dry (it is preferable to prepare and stain two or three smears simultaneously). Study the stained slides under low and high power objectives, and choose the best-stained for the study of morphology of white cells and differential leuco-

Table 5.1 Appearance of White Blood Corpuscles in a Blood Film

Feature	Neutrophils	Eosinophils	Basophils	Mono- cytes*	Lympho- cytes**
Diameter	12-14 μ m	10-15 μ m	10-15 μ m	12-18 μ m	7-10 μ m (small) 11-15 μ m (large)
Cytoplasmic granules	Fine pinkish blue, ground-glass appearance	Coarse, brick-red to orange	Coarse, deep purple, fill the entire cell	Pale, grey-blue, plentiful	Clear, slaty-blue
Nucleus	2-6 lobes, connected with chromatin threads	Usually 2 lobes connected by a thin strand, seen clearly	Irregular, may be S-shaped, not clearly seen	Eccentric, often kidney-shaped or deeply indented	Round, deeply stained
Per cent T.L.C.	40-60	1-4	0-1	6-8	20-40 (both)
No./cmm	2000-7000	10-400	0-100	500-800	1500-3000

*When the monocyte has a round nucleus, the relative amount of cytoplasm and its frosty nature helps in distinguishing it from a large lymphocyte.

**The nucleus almost completely occupies the cell and only a rim of cytoplasm is visible.

cyte count. (5) Place the slide on the fixed stage and put two drops of cedar wood oil on the stained smear at a point about 2 cm from the start of the film. Raise the body tube and swing the oil-immersion lens into position. *Watching from the side*, bring

the objective down slowly till it just enters the oil ; use the fine adjustment for final focussing of cells. (6) Move the slide slowly towards the other end and as you encounter a particular white cell, identify it and put a mark against it as shown in "Observations and Results". As you approach the end of the smear, move two fields down and scan the film in the opposite direction by manipulating the mechanical stage. Repeat this process and count 200 white cells (400 for good results). When the count is concluded, calculate the percentage of each type of cell in the total count.

Identification of white blood cells and their normal range :
Table 5.1 describes how the white cells appear in a blood film stained with Leishman's stain.

Observation and results : Different types of cells are placed in groups of five, the horizontal stroke representing the fifth cell. Record your count as shown below—(100 cells were counted in this example).

The differential leucocyte count (DLC), in this example is commonly expressed as 62, 28, 7, 2, 1.

For report—each type should be mentioned, followed by its percentage. While counting the white cells, a rough estimate of the total count can be made depending upon whether the cells appear more frequently or are sparsely populated amongst the red cells. The morphology of normal and abnormal types of red cells and premature white cells can also be studied. The chromatin of malarial parasite is stained red and the cytoplasm blue. The stain can also be used for bacteria and other micro organisms.

Discussion. Students often confuse the precipitated granules of the stain with platelets ; the granules are deep blue, rounded, of uniform size and cover the entire smear unlike platelets which are seen in groups of 2-10 and are oval with pink purple granules. The white cells assume a round shape on the slide due to surface tension ; in the circulating blood and tissue cultures, however, they manifest active amoeboid movements.

Functions of different types of granulocytes. The leucocytes fall into two major categories viz., those with prominent, stainable cytoplasmic granules, the granulocytes (or polymorpho-nuclear leucocytes—PMNs) and those without granules, the agranulocytes. Three types of cells comprise the granulocytic group, depending upon the granules binding acidic dyes (eosinophils or acidophils), basic dyes (basophils), and neutrophils in which the granules stain lightly with both dyes by a different type of reaction.

Neutrophils : They are actively phagocytic. The granules have many hydrolytic enzymes and appear to be lysosomal in character. Phagocytosis is preceded by a series of steps which include opsonization, chemotaxis, ingestion and degranulation. In the process there is formation of hydrogen peroxide, hypochlorite, hydioxyl radicals and singlet oxygen—all being strongly bactericidal.

Eosinophils : They increase in number in allergic reaction and in parasitic infestations. They release, at the site of allergic reaction, some enzymes that can inactivate the mediators of allergic response. Arylsulphotase B can inactivate *slow reacting substance* (SRS), an agent responsible for severe bronchospasm, and histaminase breaks down histamine, a substance that causes local oedema. Eosinophil granules have been shown to contain a protein, *major basic protein*, which can apparently damage certain parasites.

Basophils or mast cells : These cells contain SRS, histamine, and heparin. The exact function of these cells is not known though they resemble tissue mast cells. They bind immunoglobulin E and they release SRS and histamine in acute allergic reactions that cause local oedema, vasodilatation and contraction of smooth muscle.

Monocytes : They are derived from the stem cell *monoblast* in the bone marrow. They are actively phagocytic and contain lysosomal enzymes and peroxidase. They remain in the circulation for a few hours only and then migrate into the tissues where they mature to form the *macrophages* of the RES. They exist in many forms, for example, the Kupfer cells in the liver, the alveolar macrophages of the lungs, and the microglia of the central nervous system. They may survive in the tissues for many days or weeks and protect the body against viruses, certain bacteria, fungi, and tumour cells.

Lymphocytes. Large lymphocytes are probably the precursors of small lymphocytes. Two functionally distinct types of lymphocytes are recognised. T-lymphocytes (thymic processed) are responsible for cellular immunity concerned with rejection of tissue transplants and tumour cells. B-lymphocytes (bursa equivalent) are concerned with humoral immunity and produce circulating antibodies in the gamma globulins of plasma proteins after differentiating into plasma cells in the lymphoid tissues. Both types of lymphocytes are present throughout life and there is a recirculation of these cells through the thymus and lymphoid tissues (intestines, glands etc.) from where they re-enter circulation via the thoracic duct.

Variations in the Number of Different Types of Leucocytes

Leukocytosis
morning h.

A. Neutrophils. (a) *Neutrophilia.* (1) Acute pyogenic infections (abscess, boils, tonsillitis, appendicitis, pneumonia, lung abscess etc.). (2) Slight increase is seen under physiological conditions like severe muscular exercise, emotional stress, food intake, pregnancy and parturition.

*Phenyl
ketonuria*
*Bile
cancer*

(b) *Neutropenia.* (1) Typhoid and paratyphoid fevers, malaria, aplastic anaemia (depression of bone marrow). (2) Drugs—chloramphenicol and some other drugs depress bone marrow. (3) Some chronic infections may fail to stimulate neutrophil production.

malnutrition, debility, irradiation

B. Lymphocytes. (a) *Lymphocytosis.* (1) Chronic infections—tuberculosis and whooping cough. (2) There are normally more lymphocytes in young healthy children but the term is not used for this as well as for lymphocytic leukaemia where the number is very high.

ALL CLL
Acute lymphocytic leukaemia

(b) *Lymphopenia* is seen in patients on ACTH and steroid therapy.

C. Eosinophils. (a) *Eosinophilia*, (1) Allergic conditions—asthma urticaria, food sensitivity, hay fever etc. (2) Pulmonary eosinophilia. (3) Intestinal infestation with round worms, tape worms and others. (4) Scarlet fever.

(b) *Eosinopenia*. (1) Acute pyogenic infections. (2) Patients on ACTH and steroid treatment.

D. Basophils. Increased number is present in chronic myeloid leukaemia, small pox and polycythaemia while they may disappear altogether from circulation in acute pyogenic infections.

E. Monocytes. Moncytosis is seen in infectious mononucleosis, malaria, kala azar and rarely, in tuberculosis and typhoid fever. The term is used when the absolute number of monocytes rises above 500/c mm.

Precautions. (1) The staining time should be confirmed from the laboratory technician as these vary with each batch of stain. (2) The undiluted stain should be watched carefully in hot weather to see that it does not become syrupy or thick from evaporation of alcohol. If the stain starts drying, a little more of the stain should be added to avoid its precipitation on the slide. (3) The alcohol should be acetone free, as the latter can cause deterioration of the stain or damage to the cells.

Questions : (1) What are the steps in preparing and staining a blood film? What are the features of well stained film ? (2) What is the composition of Leishman's stain and what is the function of each constituent ? (3) Why is the stain diluted after 2 minutes and not earlier ? (4) Which objective lens will you use for examining the blood film ? (5) Classify leucocytes and describe the morphological features of each type. What are their functions ? (6) Name the conditions in which each type of WBC increases in numbers ? (7) What is Cooke-Arneth count ? What does a shift to the left signify ? (8) What other information can you derive from examining a blood film ? (9) What is peroxidase reaction and which type of white cells show a positive test ?

*Experiment No. 5.12***COOKE-ARNETH COUNT**

The lobes of the nuclei of granulocytes are connected by fine chromatin threads and are never completely separate. Neutrophil polymorphs are grouped into five classes or stages according to the lobulation of the nuclei (young cells have fewer lobes). *Stage I*—the nucleus is shaped like the letter *C*, the two lobes being joined by a broad band of chromatin material; *Stage II* the nucleus has two lobes connected by the chromatin filaments; *Stage III*—cells have a trilobed nucleus; *Stage IV*—the nucleus has four lobes; and *Stage V*—cells have 5 lobes or more. Sometimes it becomes impossible to place a cell in its proper stage because of difficulty in counting the number of lobes due to folding of the nucleus. Under such conditions, the cells is placed in the next lower category.

Prepare and stain a blood film and count 100 neutrophils, noting the number of lobes of the nucleus in each cell. Record your count as in DLC and calculate the percentage of each stage. Compare your results with the normal values.

Normal count. The ranges for each stage are : *Stage I* : 5-10; *Stage II* : 20-30 ; *Stage III* : 40-50 ; *Stage IV* : 10-15 and *Stage V* : 3-5 cells.

Clinical significance. Neutrophils enter the circulation mostly as bilobed cells, the number of lobes increasing to five or more by the end of their short life span. In acute pyogenic infections, there is a shift of the count to the left, indicating an active response by the bone marrow in forming and releasing large numbers of young cells into the circulation as a defence reaction against the invading bacteria. In physiological leucocytosis, there is no shift to the left, which indicates mobilization of leucocytes from blood stores (spleen and other viscera) but no new formation of cells.

Experiment No. 5.13

MORPHOLOGY OF RED CELLS

A thin and well-stained blood smear is essential for a proper study of the morphology of normal and abnormal red cells. The cells are studied best in the area between the tail and the thicker head of the smear, away from the edges i.e., where the cells lie just near their neighbours without overlapping. The points to be noted concerning normal mature cells are : (1) *Size and shape.* There is a moderate variation in the diameter of rbc (average $7.2 \mu\text{m}$). Most cells are round, but a small percentage may be slightly oval. (2) *Staining.* Cells of normal subjects are stained a pink colour with Leishman's stain. The staining is deeper at the periphery and gradually lessens towards the centre of the cell ; this area of central pallor occupies less than $1/3$ of the cell diameter and is due to the biconcave shape of the red cell.

Reticulocytes. These are juvenile red cells and contain the remnants of the basophilic ribonucleoprotein which was present in greater amounts in the cytoplasm of the nucleated precursors. This material appears either as a precipitate of granules, or in the form of interlacing filaments. Reticulocytes do not contain nuclei.

Abnormal red cells. Alterations in the size, shape, structure and fragility of rbc is frequently seen in various types of anaemias where erythropoiesis is abnormal. The following terms express some of the abnormal morphological states :

- (1) Anisocytosis—abnormal variation in the size of rbc.
- (2) Microcytosis.
- (3) Macrocytosis.
- (4) Poikilocytosis—cells have many nondiscoid shapes (oval, crescent, sickle and flask).
- (5) Polychromatophilia—this is the bluish tinge in the red cells due to their youth.
- (6) Punctate basophilia—bluish granules in rbc; commonly seen in lead poisoning and in severe anaemias.
- (7) Cabot's rings and Howell-Jolly bodies—these are the remnants of the nuclei which persist in the cells in some anemias.
- (8) Sickle cells—the shape resembles a sickle and is due to the presence of abnormal haemoglobins which alter the shape of cells due to their own abnormal configuration. The nucleus may appear as a central blue dot giving the cells an appearance of

target cells. The cells have low osmotic fragility. (9) Spherocytosis—the cells are more spherical, smaller in diameter, appear more dense and are osmotically more fragile. Such cells are seen in congenital haemolytic anaemias where the cells are defective though the exact enzymatic fault is not known.

Demonstration slides. Stained slides showing reticulocytes and abnormal morphology of red cells (obtained from the pathology department) will be set-up on the demonstration table. Examine and compare these slides your own blood film. Note the descriptions listed on the cards beside the microscopes and enter these in your note-book.

Experiment No. 5.14

PEROXIDASE REACTION (PEROXIDASE STAIN FOR WBC)

A thin blood film is spread as before and stained with peroxidase stain. The reaction helps in distinguishing immature cells of myeloid series from lymphoid series in cases of leukemias.

Solution 1. 0.3 g benzidine dissolved in 99 ml of ethyl alcohol with 1 ml of saturated (36 per cent) solution of sodium nitroprusside.

Solution 2. Six drops of hydrogen peroxide (20 vols) in 2-5 ml of distilled water. This solution is prepared on the day of the test.

Procedure. (1) Put 10 drops of solution 1 on a thin and dry blood film and allow it to remain on the slide for one minute and a half. (2) Add five drops of solution 2 directly on the slide and wait for four and a half minutes. Wash thoroughly for 12 minutes and allow to dry. (3) Counterstain with Leishmans stain in the usual way and examine under oil immersion. (4) Large dark blue granules are seen in myelocytic cells in general (the monocytes may show a sprinkling with fine granules). Count

200 leucocytes, estimating the percentage of typical peroxidase-positive and peroxidase-negative cells. Compare the result with differential leucocyte count.

Discussion. Peroxidase staining depends on the presence of an oxidizing enzyme in the cytoplasm of myeloid cells. Eosinophils show deeper blue granules whereas basophils usually show no granules. Oxidase granules are thus found in granulocytes, myelocytes and myeloblasts. Very young myeloblasts do not take the stain. Lymphoid series are peroxidase-negative.

Demonstration slides. Stained blood and bone marrow smears of leukaemic patients (obtained from pathology dept.) showing immature cells of myeloid and lymphoid series will be focussed on the demonstration table. Note the descriptions listed on the cards beside the microscopes and enter these in your note-books.

Experiment No. 5.15

ABO BLOOD GROUPING (SYN : BLOOD TYPING)

Principle. The red cells contain different types of agglutinogens and plasma contains agglutinins. In order to determine the blood group of a subject, the red cells are allowed to react with sera containing known agglutinins.

Apparatus and reagents. (1) Glass slides. Droppers, Normal saline. (2) *Anti-A serum* (contains α or alpha agglutinins ; obtained from a subject with blood group B). (3) *Anti-B serum* (contains β or beta agglutinins ; obtained from a subject with blood group A). These sera are available commercially, in sealed bottles (2.0 and 5.0 ml) and should be used because of their high titre of agglutinins (the anti-A serum is tinted blue and anti-B serum—yellow, for quick identification). Sera obtained from the blood bank may be used if these are not conveniently available.

Procedure. (1) Divide the glass slide into two halves with a glass marking pencil and mark these areas A and B in the corners. Place two drops of Anti-A serum on the slide in the centre of area marked A and two drops of Anti-B serum on the area marked B. (Use separate droppers for this purpose).

(2) Get a finger prick and when a good-sized drop of blood appears, place the finger tip over the open end of a small glass tube containing about 3.0 ml of normal saline. Invert the tube once so that a small amount of blood is transferred to the saline. You have now a suspension of red cells in normal saline.

(3) Place a drop of saline, containing red cells, on Anti-A serum and another on Anti-B serum. Hold the slide from one end and tilt and rotate it in all directions, avoiding mixing of the two sera. Wait for 6-8 minutes and then make your observations.

(4) Inspect the sera first with the naked eye to see whether any clumping and agglutination has taken place or not. Confirm under the microscope.

Observations and results. If any clumping and agglutination has occurred it is usually visible with the naked eye as dark reddish clumps of different sizes. If the number of rbc is very high, they may simply give an impression of agglutination, that is why microscopic confirmation is essential. The agglutinated red cells form clumps of different sizes and possess a dark reddish colour due to haemolysis of red cells. If there is no agglutination, the red cells appear grouped together in masses but without any clumping, agglutination or haemoglobin liberation. Determine your blood group by consulting the following table.

TABLE 5.2 : The ABO Blood Grouping

Blood group of subject	Anti-A serum alpha agglutinins (serum from group B subject)	Anti-B serum beta agglutinins (serum from group A subject)	Agglutinins present in the subject's serum
A	+	-	(beta)
B	-	+	(alpha)
AB	+	+	None
O	-	-	Both

Symbols : + denotes agglutination and

- denotes absence of agglutination (no agglutination)

Discussion. The human red cell membranes contain different types of antigens called agglutinogens which are glycoprotein in nature ; the best known are A, B and O (Landsteiner terminology). Human beings can be divided into four groups; A, B, AB and O, depending on the presence or absence of these antigens. These are also present in many tissues and juices of the body (liver, lungs kidneys, pancreas, salivary glands, saliva, semen, amniotic fluid etc.). Group A has two subgroups : A_1 and A_2 ; there are thus 6 blood groups— A_1 , A_2 , B, A_1B , A_2B and O. Most Anti-A sera contain two antibodies, anti-A (a) and anti- A_1 (a_1) ; anti-A(a) agglutinates red cells of all the sub-groups i.e., A_1 , A_2 , A_1B and A_2B , whereas anti- A_1 (a_1) agglutinates only A_1 and A_1B red cells. This implies that some subjects of blood group A have an additional agglutinogen called A_1 ; thus in blood group A_1 the red cells have both A and A_1 agglutinogens whereas in blood group A_2 there is only the A agglutinogen.

Blood group O does not mean that the red cells have no agglutinogen. There is a group specific substance O which does not normally act as an antigen so there is no corresponding agglutinin. Group O cells are not agglutinated by α and β agglutinins; this group is, therefore, called 'universal donor'. Very rarely, and after repeated transfusions with O group cells, an agglutinin (anti-O) may be produced. AB group is 'universal recipient' as there are no agglutinins present in the plasma of these subjects.

Other Blood Group Systems

Besides ABO blood groups, other group systems are M, N, S, P, C, E, Lutheran, Kell, Duffy and Kidd Agglutinogens for these have been demonstrated but the corresponding antibodies in the serum are generally not present (except in the P system).

Rh groups. Rh factor, first discovered in rhesus monkey, is present in 80-85 per cent of humans. It is actually a system of many antigens and antibodies. Rh+ve subjects have the Rh antigen in the red cells but no Rh antibodies. Rh-ve subjects have neither Rh antigens in the red cells nor Rh antibodies in the plasma; the antibodies may, however, appear during pregnancy,

and after repeated transfusions of Rh—ve persons with Rh+ve blood.

The most common Rh antigen is D, its antibody being anti-D. A complication due to "Rh incompatibility" is sometimes seen when an Rh-negative mother carries an Rh-positive foetus. At the time of delivery small amounts of foetal blood leak into the maternal circulation. As a result some mothers develop high levels of anti-Rh agglutinins during the period following delivery. During the next pregnancy, the antibodies from the mother cross the placenta into the foetus where they can cause hemolysis and other effects—a condition called erythroblastosis foetalis (hemolytic disease of the newborn). If the hemolysis is severe, the foetus may die, or it may be born with severe anaemia, jaundice and oedema. The first child is usually normal (provided the mother has not received Rh-positive blood transfusions before) but complications may arise in subsequent pregnancies. The effects of Rh incompatibility depend on the titre of antibodies in the mother.

It may be noted that the maternal response against Rh antigen does not occur in most cases and a majority of Rh +ve offspring of Rh —ve mothers are normal. ABO incompatibility between mother and foetus almost always confers protection against Rh immunization. Finally, injection of IgG anti-D antibody serum in a D-negative mother after the delivery of a D-positive offspring can greatly reduce the incidence of haemolytic disease of the new born during later pregnancy.

Importance of Blood Grouping

1. **Blood transfusion.** Grouping and cross-matching is always done before blood transfusion. Red cells of the donor are treated with the serum of recipient and the cells of the recipient with the serum of donor. Normally, the plasma of the donor is highly diluted in the blood of the recipient and its effect on the cells of the recipient is negligible. *What is important is the reaction between the plasma of the recipient and the cells of the donor.*

Indications for blood transfusion. The most common indications for blood transfusion are—decreased blood volume following haemorrhage, treatment of anaemia especially before surgical operations, and to supply platelets in patients suffering from purpura. Blood transfusion, even after proper grouping and

cross-matching for ABO and Rh systems, is not entirely free from some inherent risks. Diseases like viral hepatitis, venereal diseases, malaria, and the recently discovered AIDS (acquired immune deficiency syndrome—a viral disease) may be transmitted to the recipient.

Transfusion reactions from mismatched blood. In the case of a mismatched transfusion the red cells of the donor blood are agglutinated and then haemolysed in the body of the recipient. Minor reactions cause shivering and fever but if the haemolysis is rapid and large, the haemoglobin level in the plasma may rise to dangerous levels. Toxic substances released from cells and from immune reactions may cause circulatory shock. The haemoglobin filtered out of the renal glomeruli may precipitate and block many of the tubules. The end result may be acute renal shutdown. The patient may die within a few days unless put on artificial kidney.

2. Paternity disputes. ABO, MNS and Rh blood grouping is used as a routine in such cases. Agglutinogens A and B are dominant, whereas O is recessive. It is possible to disprove parentage but impossible to prove parenthood (*See Table 5.3*).

TABLE 5.3

TABLE 5.3 : Inheritance of the ABO System Blood Groups

If the child's blood group is	Parents must have given it	So if mother was	The father could not have been
A	(A+B) or (A+A)	B or O	B or O
B	(B+O) or (B+B)	A or O	A or O
AB	A+B	no matter which	O
O	O+O	no matter which	AB

3. Medicolegal use. It is first confirmed whether the red stains on the clothing etc. are blood or not, by preparing haema-

tin crystals. Blood stain is extracted from the dried clothing, using normal saline and blood grouping of the sample can then prove or disprove the claims of a victim.

4. Susceptibility to various diseases. O group subjects are said to be more susceptible to peptic ulcer. Blood group A is more frequently encountered in carcinoma of stomach, and to some extent, diabetes mellitus.

Note. Along with other hereditary traits, the difference in blood groups is quite distinct in people of different ethnic groups. The British and West Europeans have a high percentage of groups O and A. Indians have a high frequency of group B.

Precautions. 1. Use separate droppers for sera and rbc suspensions.

2. The two sera should not be allowed to mix on the slides.

3. You must wait for at least 15 minutes before confirming agglutination with naked eye and later with the microscope.

Questions. (1) What is the physiological basis of blood grouping ? What is Landsteiner's law? Is it applicable to all types of blood grouping ? (2) What is the difference between rouleaux formation and agglutination ? How will you confirm that agglutination has taken place ? (3) What are blood group-specific substances ? Are they present in the RBC only ? (4) What is meant by a 'universal' donor and a 'universal' recipient ? (5) What is Rh factor ? Can group O Rh—ve blood be given to a person of any other blood group ? (6) Should a Rh —ve female be given Rh + ve blood transfusion at any age before menopause ? Explain your reasons. (7) Why is the reaction between donor's cells and recipient's plasma taken into consideration rather than the other way around ? (8) What is the physiological basis for indications of blood transfusion ? How will you ensure complete safety against mismatched transfusion ? What complications will occur if mismatched blood has been given ? (9) Can a group O child have a group AB parent ? (10) What can be the group of a child if both parents belong to group AB ?

*Experiment No. : 5.16***BLEEDING TIME, COAGULATION TIME,
CLOT RETRACTION TIME, CAPILLARY
FRAGILITY TEST AND PROTHROMBIN TIME****A. Bleeding Time**

If a prick is given in the skin with a needle, bleeding occurs and continues for some time and then stops. The time elapse between skin puncture and the arrest of bleeding is called *bleeding time*.

I. 'Duke' bleeding time. Get a deep skin puncture on the tip of the finger under sterile conditions and note the time. Remove the drops of blood every 15 seconds by touching the skin gently with a filter paper, absorbing the drops of blood along its edge and numbering them 1 onwards. Note the time when there is no trace of blood on the filter paper i.e., when bleeding has stopped. Count the spots of blood and express the bleeding time in minutes and seconds. Normal bleeding time=1-4 minutes.

II. A more accurate methods is to get a finger prick and dip the finger in a beaker containing normal saline at 37°C. The drops of blood are seen falling to the bottom of the beaker in a continuous slow stream. Note the time when the bleeding stops. Normal BT=2-6 minutes.

III. 'Ivy' bleeding time (haemostasis bleeding time method).
(1) Apply a blood pressure cuff on the arm and raise the pressure to 40 mm Hg and maintain it at that level. (2) Make a skin puncture 2 mm deep on the anterior surface of forearm after cleaning the area with alcohol and note the time of puncture. (3) Absorb blood with a clean filter paper by gently touching the skin puncture every 15 seconds until the bleeding stops. Normal BT with this method is 2 to 6 minutes.

B. Coagulation Time

If blood is removed from the body and kept in a glass capillary tube or a test tube, it coagulates, forming a jelly-like mass.

Time elapse between the withdrawal of blood and clot formation is the *coagulation time*.

I. Capillary blood coagulation time. (1) Get a deep skin puncture on the finger tip and discard the first 2-3 drops of blood. (It is essential to prevent admixture of blood with tissue fluids). (2) Fill a chemically clean and dry glass capillary tube by dipping one end in the drop of blood. (Blood will fill the tube by capillary action). Note the time. (3) Break off a small piece (about one cm) of the tube from one end and repeat this every thirty seconds. End point is when fibrin threads ('rope' formation) of the blood will span a gap of 5 mm between the broken ends. Normal coagulation time is from 2 to 5 minutes. The capillary tube is kept between the palms (no rotating) or one may blow on it at intervals (the purpose being to keep the blood near the body temperature).

II. The 'drop' method is less accurate. A skin puncture is made and a large drop of blood placed on a clean and dry glass slide. A pin is drawn through the drop every 30 seconds, noting the time when fibrin threads adhere to the pin and move with it out of the drop of blood. Time elapse between placing the blood drop on the slide and formation of fibrin threads is the coagulation time. Normal=2-4 minutes.

III. Lee and white test tube method. This is the most accurate of the routine methods and requires venous blood. (1) Rinse three small test tubes with normal saline and allow the saline to drain off fully. Place these tubes in a small metal rack and transfer it to a water bath placed on a tripod stand, with a burner below it. Maintain the temperature of water around 37°C. (2) Draw 5 ml venous blood under sterile conditions and note the time when blood begins to flow into the syringe. Remove the needle from the syringe and eject about 1.5 ml blood into each tube. After about one minute take out the first tube and tilt it to 45°; repeat at 30 second intervals, until the tube can be inverted without spilling blood. Repeat this process on the second tube and note the time when coagulation has taken place. The third tube acts as a check and is not disturbed. The time interval between the moment of entry of

blood into syringe and the moment when the second tube can be inverted without spilling its contents is the coagulation time. Normal coagulation time is 4-8 minutes. If the test is done at room temperature, the time is 5-15 minutes.

Repeated tilting hastens coagulation that is why three tubes are employed, the third tube serves as a check on the end-point observed in the second tube. It can be confirmed by tilting the third tube. This method is more reliable than the skin puncture method as there is no admixture of blood with tissue fluid which contains tissue thromboplastin. This is the method of choice in patients on heparin treatment where a clean venipuncture is essential.

Coagulation time is prolonged in many diseases and syndromes. Afibrinogenemia, liver diseases, obstructive jaundice, deficiency of various factors as in hemophilia and christmas disease, are some of the examples. In these conditions, the bleeding time is normal.

C. Clot Retraction Time

Draw 5 ml of venous blood, remove the needle and eject the blood into a test tube. Keep the tube in a vertical position in an incubator at 37°C. Clotting occurs in a few minutes and the blood clot shrinks (retracts) over a period of time, separating itself from the walls of the tube leaving behind a straw-coloured serum. Notice the degree of retraction of the clot after 1, 12, 24 and 48 hours. Note if there is any digestion of the clot or discolouration of serum. Normally, complete retraction of the clot occurs in 24 hours. It is prolonged when the platelet count is low. The cause of clot retraction is the adherence of platelets to the fibrin threads which in turn shorten and retract.

D. Capillary Fragility Test

This test is done for assessing the fragility status of the capillaries. It may also demonstrate latent purpura.

Procedure. (1) Mark a circle, one inch in diameter, on the flexor surface of the forearm. Using blue ink, mark any pink purple or yellow spots within this circle. (2) Apply a blood

pressure cuff on the upper arm. Inflate it and keep the mercury level midway between systolic and diastolic pressures for 15 minutes. Appearance of more than ten new haemorrhagic spots (petichae) is a positive test, which may occur with thrombopenic purpura (platelet counts below 70,000/c mm), non-thrombopenic purpura and scurvy.

E. Prothrombin Time

Prothrombin is an important factor in the coagulation of blood. For the determination of prothrombin time, thromboplastin (obtained from dehydrated brain of rabbit) and calcium are added to citrated plasma and its clotting time is noted.

Solutions and reagents required. (1) Calcium chloride solution 1.1 per cent. (2) Sodium citrate solution, 3.8 per cent. (3) Thromboplastin suspension. (4) Centrifuge tube 5 ml capacity. (5) Four test tubes 1 cm x 5 cm.

Procedure. (1) Take 0.5 ml of 3.8 per cent sodium citrate solution in the calibrated centrifuge tube. Draw 5 ml of venous blood by a clean venepuncture and deliver 4.5 ml blood to the centrifuge tube. Mix the contents thoroughly by repeatedly inverting the tube gently and centrifuge it for 15-20 minutes. Transfer the citrated plasma to a clean dry tube. (2) Put 2.0 ml each of citrated plasma, thromboplastin suspension and calcium solution in separate test tubes placed in a metal rack, in a water bath at 37°C. (3) A separate tube is kept half submerged in the water bath. Deliver 0.2 ml of citrated plasma to this tube and add 0.2 ml of thromboplastin suspension. After about 2 minutes, add 0.2 ml calcium solution, starting the stop watch simultaneously. (4) Tilt the tube to ascertain the clotting of plasma. You may agitate the plasma with a small glass rod bent at the end. Note the time when the first fibrin threads are pulled out.

Normal prothrombin time is 15-20 seconds. When the technique is used in clinical practice to control anticoagulant drug therapy or in a haemorrhagic disease, blood from a normal person is used as a control because the potency of thromboplastin solution may vary. Excess of calcium is used to provide free calcium ions which had been removed by the citrate. Bleed-

ing tendency is present when the plasma prothrombin level falls below 20 per cent of normal (normal prothrombin level 30-40 mg per cent). Prothrombin level is low in vitamin K deficiency and various liver or biliary diseases.

In patients on anticoagulant therapy, the prothrombin time is determined at intervals of 3-5 days. When the dose of anti-coagulant has been stabilised, the time may be determined at longer intervals.

Discussion. The student must differentiate between coagulation time and bleeding time. One is commonly affected without a change in the other in different diseases.

Coagulation of blood is a complex process in which the soluble protein fibrinogen is converted into insoluble fibrin threads by the action of thrombin. In the circulation, the blood is kept in a fluid form by the special physical properties of the lining endothelium, and the natural anticoagulants like fibrinolysis, heparin etc. present in the blood. The factors involved in coagulation are : I. Fibrinogen. II. Prothrombin. III. Tissue thromboplastin. IV. Ionised calcium. V. Labile factor, Proaccelerin, Accelerator globulin. VI. None. VII. Stable factor, Serum prothrombin conversion accelerator (SPCA), Autoprothrombin I, Proconvertin. VIII. Antihaemophilic globulin (AHG), Antihaemophilic factor (AHF), Platelet co-factor I, Antihaemophilic factor A. IX. Christmas factor (CF), Antihaemophilic factor B, Plasma thromboplastin component (PTC). X. Stuart-Prower factor. XI. Plasma thromboplastin Antecedent (PTA), Antihaemophilic factor C. XII. Hageman factor, Contact factor. XIII. Fibrin-Stabilizing factor (PSF) Laki-Lorand factor.

Haemostasis. The term refers to the process by which bleeding from damaged blood vessels is arrested to maintain the blood within the blood vessels and in a fluid state. It is a complex process, involving (a) plasma clotting factors, (b) platelet adhesion and aggregation, (c) the reactions of the blood vessels, and (d) the fibrinolytic system which modifies the other three.

of the clear plasma above the red cells, in millimetres (the upper level of red cells column is usually sharp and clear). Express your results in mm 1st hour Wintrobe. Normal values ; Males= 2-8 mm 1st hour ; Females=2-10 mm 1st hour (ESR is not expressed as mm per hour).

B. Westergren's Method

The Westergren tube is open at both ends (Fig. 5.9) and is graduated in mm from 0 to 200, with a bore diameter of 2 mm. A sterile solution of 3.8 per cent sodium citrate is used as the anticoagulant.

Procedure. (1) Clean the antecubital area with alcohol and allow it to dry. Take 0.4 ml of 3.8 percent sodium citrate solution in a 2.0 ml syringe. Puncture the skin and the vein and draw blood directly into the syringe by drawing out the plunger to the mark 2.0 ml mark (1.6 ml blood and 0.4 ml citrate solution). Remove the needle and expel the blood into a small bottle. Mix the contents gently by rotating the bottle on the surface of table. (2) Suck blood into the Westergren tube (keeping the tip of the index finger gently over the upper open end) and allow it to empty into the bottle twice, finally sucking the blood to the 0 mark. Keeping the finger over the open end, transfer the tube to the Westergren stand. Place the lower end of the tube firmly over the rubber cork and fix its upper end under a spring fitted to the stand which will hold and keep the tube in a vertical position without leakage of blood from its lower end. (3) Allow the tube to remain in this position for one hour at the end of which, take the reading in millimetres of the clear plasma column above the red cells.

Normal values ; Males=3-5 mm 1st hr ; Females=7-12 mm 1st hr.

The significance of the results is the same with both methods, though the normal values vary slightly with the method employed. The diameter of the bore of the tube (if not less than 2 mm) has no effect on ESR but inclination from the vertical gives false high values. High values are encountered in anaemia, especially if the count is less than 3.5 million/c mm. Charts are available for

applying a correction factor. The sedimentation rate is not affected by room temperature, food intake, muscular exercise, the age and subject's body temperature as such.

Discussion. Much significance was attached to raised ESR in the past for diagnostic and prognostic purposes in various diseases, especially tuberculosis of the lungs. Greater facilities for radiological examination of the chest in lung diseases has, however, lessened its importance. Nevertheless, ESR is determined as a routine laboratory investigation to exclude organic diseases.

Constant movement of blood keeps the red cells fairly distributed in the circulation but when it is kept in an upright state in a tube, the cells tend to settle down due to a greater tendency to rouleaux formation. Alterations in plasma protein patterns (ESR is greater in plasma than in serum) and products of tissue destruction and inflammation are known to increase the speed of piling up of red cells (the ratio of surface area to mass in a rouleaux is important). These alterations possibly affect the electrostatic charges on the red cells, increasing their tendency to form rouleaux at a faster speed.

ESR is increased in. (1) Acute inflammatory conditions, acute episodes in chronic diseases, and acute rheumatic fever. (2) Lung disease—tuberculosis, emphysema, abscess. (3) Bone diseases—tuberculosis, rheumatoid arthritis, osteomyelitis. (4) Malignancies and collagen diseases. (5) Anaemias. (6) Pregnancy (physiological).

ESR is reduced in. (1) Polycythaemia of infants (physiological) (2) Polythemia due to hypoxia (high altitude, lung diseases and congenital heart disease). (3) A fibrinogenemia.

Precautions. The test is quite sensitive, and if the procedure is not followed strictly, the results obtained will be invalid and useless. (1) Proper anticoagulant must be used, and the tube employed should be clean and dry. Clotted and/or haemolysed blood should not be used, and the test done within 2-3 hours of the withdrawal of blood. (2) The tube must be kept absolutely vertical. (3) The haematocrit must be checked and correction

applied, if necessary. (4) Express the results as millimetres 1 st hour and *not* per hour.

Questions. (1) What is the specific gravity of RBC, plasma, and blood ? Why do the RBC settle down when blood, to which an anticoagulant has been added, is allowed to stand upright in a tube ? (2) Name the methods employed for the estimation of ESR. Which method possesses an advantage over the other ? (3) Name the conditions in which the ESR is raised and the reason why it is raised ? What does a raised ESR signify ? Is the ESR decreased in any conditions ?

Experiment No. 5.18
EOSINOPHIL COUNT

Blood is diluted 10 times in a wbc pipette using eosinophil solution, which lyses the red cells and white corpuscles except eosinophils. The cells are then counted in a haemocytometer. The composition of the diluting fluid is—5.0 ml of 1 per cent eosin solution in water, 5.0 ml acetone (analytic) and 90.0 ml of distilled water.

Procedure. (1) Get a finger prick under sterile conditions. Discard the first 2-3 drops and draw blood into a wbc pipette to mark 1, followed by the eosin solution to mark 11. Mix the contents of the bulb thoroughly for 30-40 seconds and put it aside for 15-20 minutes for proper lysis and staining. (2) Mix the contents of the pipette again for 30 seconds, charge the chamber as before and count the eosinophils in the 4 groups of 16 squares each. (3) The volume of the squares is known and the dilution employed is 1 : 10. The number of eosinophils in undiluted blood can be easily calculated. The normal absolute count of eosinophils per c mm of blood has a range of 10-400.

Eosinophil count is indicated in patients having a large percentage eosinophils in differential white cell counts. A patient suffering from pulmonary eosinophilia may have a TLC of 16,000 cmm and the differential count may show 15 per cent eosinophils. It is under such conditions that the absolute eosinophil count is relevant. The use of ACTH and adrenal steroids in clinical medicine has also directed attention to the determination of the absolute number of eosinophils in circulating blood.

Eosinophilia is seen in. (1) Pulmonary eosinophilia. (2) Allergic conditions—asthma, nasobronchial allergy. (3) Intestinal infestation with worms (round worms, hook worms etc).

Eosinopenia is seen in. (1) Patient on ACTH and steroid therapy. (2) Sometimes in acute pyogenic infections.

*Experiment No. : 5.19***PLATELET COUNT****Direct Method**

The blood is diluted 200 times in a rbc pipette with Reese-Ecker fluid and the stained platelets are counted in a haemocytometer. The composition of the diluting fluid is—Sodium citrate—3.8 g, Brilliant cresyl blue—0.5 g, Formaline—0.2 ml and Distilled water—100 ml. The dye stains the platelets, formaline prevents fungal growth and sodium citrate makes the fluid isotonic with blood.

Procedure (1) Draw freshly filtered platelet solution to the 0.5 mark in the rbc pipette. Wipe the tip and then draw blood from a finger prick to the 0.5 mark so that the platelet solution reaches the mark 1. Wipe the tip again and fill the pipette with platelet fluid to the mark 101. Mix the contents for 1-2 minutes. (2) Discard the first 3-4 drops and charge the counting chamber with the diluted blood. Count the platelets in 5 groups of 16 squares each (as for red cell count) and calculate their number in undiluted blood.

Indirect Method

(a) Place a drop of 14 per cent magnesium sulphate solution on a clean dry finger tip and get a prick through this drop.
 (b) Blood mixes with the solution and prevents clumping and disintegration of platelets. Make a blood smear and stain with Leishman's stain. Count the platelets per 1000 red cells. Determine the red cells count separately from a fresh drop of blood. Calculate the platelet count from total rbc count obtained. Usually, there is 1 platelet to 20 red cells, e.g., if there are 80 platelets per 1000 rbc and the count is 5 million/c mm, there would be $80 \times 5,000$ i.e., 400,000 (4 lakhs) platelets. Normal count = 2.5—5 lakh/c mm blood. The ratio of platelets to rbc is called *platelet ratio*. Normal ratio is 1 : 18 to 1 : 20.

Discussion. Platelets are small, membrane-bound, non-nucleated granular bodies varying from 2 to 3 μm (average 2.5 μm) in diameter. They are oval or disc shaped, 0.5 μm thick, with a volume of about $5.8\mu^3$ and are formed from megakaryocytic cytoplasm (each produces more than 1000) in the bone marrow from where they enter the circulation. The cytoplasm is slightly condensed in the centre, giving the impression of a nucleus. Platelets are rich in serotonin, adrenalin, ATP, Ca^{2+} , K^+ , enzymes and clotting factors. They possess an extreme surface instability and throw out long pseudopodia, and rapidly stick to and spread around other platelets, collagen fibres or objects like glass. They obtain their energy by oxidative phosphorylation and glycolysis. Actinomyosin-like proteins discovered in them are responsible for clot retraction, as the platelets adhere to the fibrin threads. Their life span is about 7-10 days. Young and old platelets are removed from the circulation, presumably for the repair of minor vascular damage. Their number is possibly controlled by "thrombopoietin" or a spleen extract fraction.

Platelet factor I is plasma clotting factor V absorbed onto these; factor II accelerates the clotting of purified fibrinogen by thrombin; platelet factor III is the phospholipid essential for clotting mentioned above and platelet factor IV has the ability to inactivate heparin. In addition to factor V, other clotting

factors are also probably absorbed, especially factors VII, X and XIII.

Variations in the number of platelets under physiological conditions are uncommon but may be found after severe muscular exercise, and sometimes at high altitudes. Thrombocytosis is sometimes seen after splenectomy. Counts towards lower range of normal may be seen in females during menstruation and in the newborns.

Thrombocytopenia. Spontaneous haemorrhages occur in joints, muscles, skin and mucous membranes. The condition is encountered in : (1) Idiopathic thrombocytopenic purpura (ITP) or essential thrombocytopathy. (2) Periodic thrombocytopenia or purpura haemorrhagica (cause unknown). (3) Bone marrow depression due to cytotoxic drugs and irradiation. (4) Bone marrow dysplasia, leukaemias, secondary deposits of cancer, and megaloblastic anaemias. (5) Infections—acute specific fevers (platelets decrease in number along with the disappearance of eosinophils from circulating blood). (6) Toxins—infections, septicemia and uremia. (7) Drugs which depress bone marrow in sensitive subjects e.g., sulpha and penicillin groups, chloramphenicol and others.

Questions : (1) What is the composition of Reese—Ecker fluid ? What is the function of each constituent ? (2) What is the site of formation and function of platelets ? (3) What biological substances are associated with platelets ? (4) What is the normal platelet ratio ? (5) Name the conditions associated with thrombocytopenia.

Experiment No. : 5.20

RETICULOCYTE COUNT

These cells are the precursors of red cells and can be stained only supravitally (staining of 'living' unfixed cells). After staining, the reticulocytes show a blue-coloured network of thread-like structures, dots or filaments.

Procedure. (1) Place one drop of 1 per cent brilliant cresyl blue in normal saline on one end of the slide. Get a finger prick and add one equal-sized drop of blood to the cresyl blue. Stir with a pin and allow the mixture of the blood and dye to remain on the slide for one minute. (2) Spread a smear, dry it, and counterstain with Leishman's stain. Examine the film under oil immersion, identify and count the reticulocytes per 1000 red cells. Calculate the percentage of reticulocytes. With a normal rate of red cell production the normal reticulocyte count is 0.5 to 2.0 per cent.

Discussion. Leishman's stain alone does not stain the reticulum inside the red cells, cresyl blue is, therefore, used for this purpose. The normal range of absolute count varies from 25000-75000/mm³ blood. These cells are young red cells but are larger and stain lightly than the mature cells. The reticular material is neither of nuclear nor mitochondrial origin. Chemically it is ribonucleic acid and can also be demonstrated in the normoblasts. It is believed to be concerned with the formation of haemoglobin. The cell membrane is sticky and is believed to be involved in regulating the release of cells into the circulation. These cells, because of their larger size and stickiness, are trapped in the spleen and undergo maturation there.

Reticulocyte response indicates increased bone marrow activity and formation of new rbc when patients suffering from deficiency anaemias are put on treatment.

Physiological variations. Increased count is seen in. (1) Newborn infants. The circulating blood contains a fair number of immature red cells, both nucleated and reticulocytes. After one year, the blood does not contain nucleated cells, and not

more than 2 per cent reticulocytes. (2) Stimulation of bone marrow under hypoxic conditions (e.g., at high altitude) raises the count.

Pathological variations are encountered in : (1) Reticulocyte response during treatment of deficiency anaemias. (2) Haemorrhage. (3) Disorders of bone marrow (nucleated rbc are also present), leukaemias, secondary deposits in malignancy and miliary tuberculosis. (4) Disorders of spleen and splenectomy. In such cases, the reticulocyte response is erratic and irregular. (5) Some chronic haemolytic anaemias. (6) Many substances like arsenic and foreign proteins produce an irregular reticulocyte reaction in an anaemic person without increasing the rbc count or Hb concentration.

From the above, it is clear, therefore, that a reticulocyte response by itself must be interpreted very critically, either as evidence of therapeutic activity of drug during treatment of anaemia, or as a prognostic sign, or for the assessment of bone marrow efficiency.

Questions : (1) Why is the mixture of blood and dye left on the slide for one minute before making a smear ? (2) Are reticulocytes normally found in the peripheral blood ? (3) What is reticulocyte response ? (4) Name the conditions in which the reticulocyte count is increased.

Experiment No. : 5.2I

OSMOTIC FRAGILITY OF RED BLOOD CELLS (SYS : OSMOTIC RESISTANCE OF RBC)

Principle. The normal red cells can remain suspended in isotonic saline solution for many hours without rupturing and releasing haemoglobin into the saline. In some diseases, the

cells are more fragile, and the ease with which they may rupture can be determined quantitatively by exposing them to various strengths of hypotonic saline solutions.

Reagents and apparatus. (1) Wooden rack. 12 clean, dry 1.0 cm × 7.5 cm glass tubes. (2) Distilled water. 10.0 ml measuring pipette. (3) 1.0 per cent sodium chloride solution (100 ml).

Procedure. (1) Place the test tubes in a rack and number them 1 to 12. Table 5.4 shows the amounts of distilled water and 1.0 per cent sodium chloride solution to be added to each tube. Use the

TABLE 5.4. Preparation of Saline Solutions for Testing the Osmotic Fragility of Red Cells

Test tube numbers	1	2	3	4	5	6	7	8	9	10	11	12
ml of 1 per cent sodium chloride solution	4.0	3.5	3.0	2.75	2.5	2.25	2.0	1.75	1.5	1.25	1.0	0.75
ml of distilled water	1.0	1.5	2.0	2.25	2.5	2.75	3.0	3.25	3.5	3.75	4.0	4.25
Tonicity (strength of NaCl in per cent)	0.8	0.7	0.6	0.55	0.5	0.45	0.4	0.35	0.3	0.25	0.2	0.15

10ml measuring pipette and mix the contents by putting a thumb on the tube and inverting it a few times. Mark the tonicity on each tube with a glass-marking pencil. (2) Draw 2.0 ml of venous blood under sterile conditions. Eject one drop into each tube successively, inverting, it with a thumb placed over the open end. (3) Set the test tube rack aside for one hour before inspecting the effects of various solutions on the red cells.

Observations and results. If there is no haemolysis, the red cells will be found at the bottom of the tube, leaving a clear saline above. If some haemolysis has occurred, the saline will be tinged red with haemoglobin. The colour of the saline will be increasingly deeper with decreasing concentrations of sodium chloride (decreasing tonicity) in the test tubes. If haemolysis is complete, the saline will be uniformly coloured throughout and there will be no red blood cells visible at the bottom of the tube. *Normally* haemolysis begins at 0.45 per cent NaCl solution and is complete at 0.3 per cent. No red cells haemolyse in solution of 0.47 per cent and above.

Record your observations as the strength of NaCl at which haemolysis begins and the tube where it is complete. If you are testing the fragility of red cells in a patient of haemolytic anemia, always check it against a normal sample of blood.

Discussion. Haemoglobin is not freely present in red cells but is bound to the structural framework proteins called ketkins. Purely mechanical agencies cannot cause liberation of haemoglobin from the cells, but many physical and chemical agents act on these cells, setting the haemoglobin free. This process of dissolution of red cells with liberation of Hb is called haemolysis. (The blood is said to be haemolysed or 'laked').

The osmotic pressure of plasma is equivalent to the osmotic pressure of 0.9 per cent sodium chloride solution. In hypertonic saline, the rbc shrink due to movement of water out of the cells. In hypotonic saline, water passes into the cells which gradually lose their biconcave disc shape and become rounded (spherocytes), the red cell membrane is stretched and ultimately bursts, pouring the haemoglobin into the saline.

In congenital haemolytic anaemia (hereditary spherocytosis), the red cells haemolyse in 0.6 per cent saline because of abnormal morphology. In these patients, intravascular haemolysis results in haemoglobinemia, and the circulating haemoglobin is excreted in urine, imparting it a red colour. Haemolysis can also result by the action of certain drugs and infections, beside

inherited disease. The tendency of rbc to haemolyse by these agents is increased by the deficiency of glucose-6-phosphate dehydrogenase (G6PD). This enzyme is required for the glucose oxidation by the hexosemonophosphate pathway, which generates NADPH. Normal red cell fragility is dependent on NADPH in some unknown manner. Deficiency of G6PD is known to be the most common human enzyme abnormality.

Questions : (1) How will you prepare normal saline ? (2) How will you determine the beginning and end of haemolysis ? How will you confirm that haemolysis is complete ? (3) Name the conditions associated with increased fragility of red cells.

Experiment No : 5.22

ESTIMATION OF PLASMA PROTEINS COPPER SULPHATE SPECIFIC GRAVITY METHOD

Small drops of plasma or serum are allowed to fall successively into copper sulphate solutions of known specific gravity and by consulting a chart, the concentrations can be determined.

Preparation of Copper Sulphate Solution

1. Stock solution of copper sulphate (specific gravity 1.100) is prepared by dissolving 159.0 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in one litre of distilled water at 25°C. Its specific gravity is checked by weighing 100 ml in a volumetric flask, against distilled water.

2. Standard copper sulphate solutions are prepared by dilution of the stock solution. 100 ml portions are prepared and kept in wellstoppered 150 ml bottles (1) THE BOTTLE NUMBERS (2) ml of STOCK SOLUTION PER 100 ml DISTILLED WATER ; (3) SPECIFIC GRAVITY-and (4) PROTEIN CONC. g per cent is given below :

(1) 14.90—1.016—3.3 ; (1) 16.80—1.018—4.0 ; (3) 18.80—1.020—4.7 ;
 (4) 20.70—1.022—5.5 ; (5) 21.79—1.023—5.8 ; (6) 22.70—1.024—6.2 ;
 (7) 23.75—1.028—7.7 ; (8) 23.70—1.026—6.9 ; (9) 25.70—1.027—7.3 ;
 (10) 26.65—1.028—7.7 ; (11) 27.60—1.029—8.0 ; (12) 28.60—1.030—8.3 ;

Once prepared, the solutions can be used for 40 to 50 estimations. Paste labels on the bottles, indicating serial number, specific gravity and protein concentration.

Procedure. (1) Arrange the bottles containing copper sulphate solutions of increasing specific gravity from left to right, in a row on the surface of a table of convenient height. (2) Start in the middle of the row of bottles. Allow a drop of plasma or serum to fall into the solution from a height of about 1.0 cm from a Pasteur pipette (the size of the drop need not be constant). The drop breaks through the surface of the solution, sinks to about 2-3 cm below the surface and its momentum is lost within 3-4 seconds. The drop of plasma then either begins to rise soon after or sinks to the bottom of the solution, or becomes stationary. Observe the behaviour of the drop during the next 15-20 seconds. If the drop continues to sink, move to the next solution of higher specific gravity; if it begins to rise, move down to a lower specific gravity solution till you come to a solution where the drop remains stationary for 15-20 seconds. Note the concentration of proteins indicated on the label. Normal plasma protein level=6.0—8.0 g per cent.

Discussion :

When the drop of plasma falls into the solution it becomes encased in a layer of copper proteinate and there is no change of specific gravity for 15-20 seconds. Within a short time the drop becomes heavier and sinks to the bottom of the solution as a precipitate.

The specific gravities of plasma, blood and erythrocytes are : 1.028—1.032, 1.055—1.062 and 1.092—1.095 respectively. The concentration of different plasma proteins and their molecular weights are : Albumin=4.0—5.5 g per cent (m.w.=68,000),

Globulins=1.5—3.0 g per cent, (m.w.=150,000) ; Fibrinogen=0.3—0.5 g per cent (m.w.=500,000) and Prothrombin=30.0—40.0 mg. per cent. Using electrophoresis, the patterns of serum proteins are : Albumin=57 per cent; $a_1=4.7$ per cent; $a_2=8.45$ per cent, $b=11.33$ per cent and gama globulin=18.52 per cent. Their absolute concentrations can be determined from total protein concentration.

The total osmotic pressure of plasma is about 5600 mmHg. The osmotic pressure of plasma proteins is only 25 mmHg, but it is this, the colloid osmotic pressure, which is concerned with tissue fluid formation. The rest of the osmotic pressure of plasma is exerted by various solutes and is equal on either side of the capillary wall, hence it has no role in tissue fluid formation.

Questions : (1) From what height the drop of plasma (or blood) should be allowed to fall into the copper sulphate solutions ? What will happen if it is dropped from a greater height ? (2) What is the normal range of specific gravity of blood, RBC, and plasma ? (3) Name the plasma proteins and give their normal concentrations. What are their functions ? How can the various fractions be separated ? (4) What is the total osmotic pressure of blood ? How much of it is contributed by the plasma proteins ? What is the importance of this osmotic pressure ? (5) Enumerate the forces involved in tissue fluid formation ? What is the effect of decreased albumin concentration on this process ?

Experiment No. : 5.23

SPECIFIC GRAVITY OF BLOOD

The copper sulphate specific gravity method is used not only for the determination of plasma proteins but also for haemo-

globin and haematocrit values. It has proved useful in screening blood donors and in emergency cases of burns who need repeated transfusions. This method was extensively used during the Second World War in assessing battle casualties requiring blood transfusions. Table 5.5 shows the amounts of stock copper sulphate solution and distilled water to prepare 100 ml portions of the solution. The procedure is the same as that for the estimation of plasma proteins. The normal specific gravity of blood is 1.055—1.062.

TABLE 5.5. Preparation of Copper Sulphate Solutions
for Specific Gravity of Blood

Bottle number	1	2	3	4	5	6	7
Stock solution in ml	49	51	54	57	59	61	64
Distilled water in ml	51	49	46	43	41	39	36
Specific gravity	1.050	1.052	1.055	1.058	1.060	1.062	1.065

Benzene-chloroform mixture method. Benzene (sp. gr. 0.877) and chloroform (sp. gr. 1.48) are mixed in a urine glass. A drop of blood is allowed to fall into the mixture and benzene or chloroform are added as indicated so that the drop floats in the middle of the mixture. The specific gravity of the mixture is then determined with a hydrometer which indicates the specific gravity of blood.

Experiment No. : 5.24

DETERMINATION OF VISCOSITY OF BLOOD

Viscometer. The apparatus is a U-shaped glass tube with a capillary bore, with one arm about 3 inches longer than the

other. The end of the long arm is expanded so that blood may be easily poured into it. The short arm has a bulb with markings 1 and 2, below and above it. Blood to which an anticoagulant has been added is poured with a narrow dropper into the expanded portion and allowed to flow down the capillary tube towards the bulb. The time taken by the blood to move from mark 1 to mark 2 is noted with a stop watch. Several readings are taken and the average is compared with distilled water. The apparatus should be kept in a vertical position throughout the determination.

The resistance to the flow of any fluid depends on its viscosity i.e., the mutual attraction of its constituent particles. The shape of the molecules, rather than the size determines the viscosity. Viscosity of blood is about 4-5 times that of water and is exerted both by the cells and plasma, each contributing about equally to it.

The viscosity of blood contributes to the peripheral resistance and helps in maintaining blood pressure. Variations in viscosity influence the load that the heart is subjected to during its contraction.

Increased viscosity is seen in—Polycythaemia, congestive heart failure, diabetes mellitus, multiple myeloma, icterus, profuse sweating when fluid intake is restricted, severe vomiting and diarrhea and leukaemias.

Decreased viscosity is seen in—anaemia, oedematous states and sometimes in malaria.

Questions : (1) Define viscosity. On what factors does it depend? What is its physiological significance? (2) What are the effects of increased and decreased viscosity of blood?

Respiratory System

INTRODUCTION

The lungs have a constant tendency to collapse, but this is prevented by the cohesive force of the thin film of fluid, which keeps the two layers of the pleura in firm contact with each other during expansion of lungs. If a small amount of air is introduced into the pleural cavity, the visceral and parietal layers separate out from each other and the lung on that side collapses. The intrapleural pressure remains negative, both during inspiration (-6 mm Hg) and expiration (-3 mm Hg) though it may be as great as -9 to -13 mm Hg at the end of a deep inspiration. The pressure becomes positive, however, under certain physiological conditions like, brief periods of straining, defecation, micturition and parturition etc. During Valsalva's manoeuvre (forced expiration against a closed glottis), the intrapleural pressure may reach $+40\text{ mm Hg}$ or more, whereas during Muller's manoeuvre (forced inspiration against a closed glottis) the pressure may become -40 mm Hg . Expiration, during normal, quiet breathing, is purely a passive process and is due to the recoil of the thoracic structures which were stretched during inspiration. Accessory muscles of inspiration and expiration are brought into play during physical exercise and in certain lung diseases. Contraction of inspiratory muscles increases the size of the thoracic cage, and as its walls move out, the lungs follow suit (against their collapse tendency); the intrapulmonary pressure falls to about -2 mm Hg and atmospheric air rushes into the lungs.

The cause of first breath after birth, is a sudden awakening of the nervous system. The presence of a surfactant, a lipid surface tension lowering agent in the fluid lining the alveoli, plays an important role in maintaining the flow of air into and out of the alveoli. The surfactant is a complex 2-phase mixture of protein and lipids, but its major constituent is dipalmitoylphosphatidylcholine (DPPC). The DPPC molecules are oriented at the air-fluid interface in the alveoli. As the alveoli become smaller during expiration, the surface tension of the fluid lining these must decrease otherwise the alveoli will collapse in accordance with the law of Laplace. Some infant mortality is due to a congenital deficiency or absence of surfactant.

Some general data. 1. Size of alveoli : 0.075—0.125 mm.
Shape : Semiglobular. Total number : 700 millions.

2. Respiratory membrane : It is $1.0\mu\text{m}$ thick and gaseous exchange occurs through it according to pressure gradients of gases. The gases diffuse through—a fluid layer containing surfactant (DPPC), epithelial cells, basement membranes of alveoli and pulmonary capillaries, and the endothelial lining of capillaries.

3. Total respiratory exchange surface area :

Average= 50 m^2 ; Range= $40-80 \text{ m}^2$

4. Minute blood flow through lungs : 5 litres at rest.

5. Minute ventilation : 6-8 litres at rest.

6. Pressures in Pulmonary Artery : Systolic=20-40 mm Hg, Diastolic =5-10 mm Hg, Pulse pressure=17-20 to 30 mm Hg, Mean Arterial pressure=12-30 mm Hg.

7. Pulmonary capillaries : Blood pressure=5-7 mm Hg, Osmotic pressure=25 mm Hg (plasma proteins). No tissue fluid formation, Diameter= $8-10 \mu\text{m}$, Length= 0.1 mm .

8. Total amount of blood in lungs : During inspiration=800-900 ml, During expiration=500-600 ml.

9. Pulmonary capillary blood volume : 60-100 ml.

10. Time for which blood stays in pulmonary capillaries : 0.3-0.7 second.

11. Time required for gaseous exchange : 0.01 second.
Exchange of gases is "time integrated".

12. Diffusion and gas exchange :

Oxygen consumption : 250 ml per minute

Carbon dioxide output : 200 ml per minute

Respiratory exchange Ratio : $\frac{\text{CO}_2 \text{ output}}{\text{O}_2 \text{ uptake}} = 0.8$

Diffusing capacity of oxygen : 20 ml/min/mm Hg
pressure difference

Maximum diffusing capacity 60 m/min/mgHg pres-
(exercise) : sure difference

Experiment No. : 6.1

EXAMINATION OF THE CHEST

1. **Rate of respiration.** If you look at the movements of chest and try to count the rate of breathing, the subject is likely to become conscious of the fact and the respiration will become irregular. Use a little deception. Put your fingers on the radial artery, giving the impression that you are examining the pulse, but watch the movements of the chest surreptitiously and count the respiratory excursions for one minute.

Average : 14-16/min Range : 12-24/min.

2. Inspect the form of chest from all sides, especially from behind and over the shoulders. Normally, the transverse diameter is greater than the anteroposterior diameter ; the normal ratio of these (the Hutchinson's Index) being 7:5. Note if there is any asymmetry, hollows or bulges.

3. Measure the expansion of the chest with a tape-measure at the level of the nipples. Normally, the chest should expand by two inches or more after a deep inspiration.

4. Look for the rhythm of respiratory movements. The rhythm is quite variable from person to person even in health and at different times of the day. Look for abnormal rhythms like Cheyne-Stokes' breathing or Biot's breathing.

5. Note the type of respiration. It may be thoracic, abdominal, thoracoabdominal or abdominothoracic. See if the subject feels any pain during respiratory movements. Note whether the type of respiration is similar or different on the two sides.

Experiment No. : 6.2.
BREATH SOUNDS

Movement of air into and out of the lungs produces sounds which can be heard with a stethoscope. Normal healthy lungs produce vesicular type of breath sounds all over the chest but can be heard to best advantage in the axillary region. Bronchial type of breath sounds are heard over diseased parts of the lungs, for example, in consolidation, tuberculosis etc. detailed description of these sounds are given in 6.14.

Experiment No. : 6.3
STETHOGRAPHY
RECORDING OF NORMAL AND MODIFIED RESPIRATORY MOVEMENTS

Principle. A stethograph (pneumograph) is tied around the subject's chest and held in position with an open link metal chain and hook. Movements of the chest cause air pressure changes in the stethograph which can be recorded on a moving paper.

Apparatus:

1. Kymograph. Cylinder with smoked paper.
2. Stethograph. It is constructed of corrugated canvas rubber, with side clips and open link chain. One end is closed and the other end can be connected to a tambour (Fig. 6.1.)
3. Brodie's tambour (modified). This is a metallic cup with a side tube and a 3" diameter rubber diaphragm mounted at the top. A capillary writing lever is attached to a small metal disc which rests on the rubber diaphragm, and the side tube of the metal cup can be connected to the stethograph by a rubber tube. With this *closed-air system* large displacements of air can be transmitted to the tambour.

4. Tap water in a cup.
5. Polythene bag : 5-6 litre capacity.

Procedure. 1. Seat the subject on a stool near the work table and tie the stethograph around his chest at a level where the movements are maximum. Adjust the position of the stethograph and the amount of air in the closed-system in order to get maximum response of the writing lever to respiration. Have the subject sitting with his back to the recording drum and allow him to relax and breathe regularly without any effort. Connect the stethograph to the tambour so that the pressure changes in the stethograph are transmitted to it. The lever will be seen to move with the movements of the chest.

2. Bring the writing lever in contact with the recording surface at a tangent and set the calibrated speed lever at slow position (2.5 mm/sec). (The movements recorded should be about an inch in height). Record the movements for 2-3 inches on the paper.
3. Ask the subject to drink water from the cup while the movements are being recorded. Follow this by a few normal movements.
4. Record the effects of coughing, sneezing and talking on respiratory movements. Indicate each event on the paper with an arrow.
5. Record some normal movements again and ask the subject to hold his breath for 30-40 seconds (voluntary apnoea). Record the chest movements during and after breath holding till the movements return to the normal pattern. (Using a stopwatch, note the time taken for movements to return to normal). You have now two parameters viz., duration of breath-holding and the time taken for the movements to return to normal. Record these in your notebook.
6. Ask the subject to breathe rapidly and deeply for 20 times. Record these tracings on the paper continuously and continue recording the effects of voluntary hyperventilation. Stop the kymograph.

7. Ask the subject to breathe into and out of the polythene bag for about 10-15 seconds (discontinue if there is much discomfort). Switch on the kymograph and record the effects.

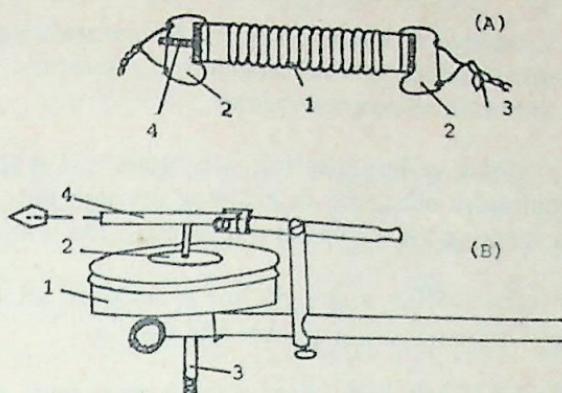


Fig. 6.1 : (A) Stethograph (pneumograph) for recording respiratory movements. 1. Corrugated rubber-canvas tube ; 2. Side clips ; 3. Open link chain : 4. Side tube for transmitting pressure changes to the tambour.

(B) Brodie's tambour. 1. metal cup ; 2. Metal disc (rest piece) resting on the rubber diaphragm ; 3. Side tube a rubber tube connects it to the side tube (4) of stethograph.

4. Writing lever.

8. Disconnect the stethograph from the tambour and ask the subject to 'do spot-running' (thighs brought to horizontal position alternately) for 50 times. Connect the stethograph again with the tambour and record the effect of exercise (a metronome may be used to set the rate for 'spot-running').

9. Record a 5 second time tracing below the graph obtained.

10. Put the title of the experiment above the tracing. Label the various events recorded and fix the graph in fixing solution after getting it signed from a teacher.

Observations and results. Observe the tracing carefully and note that—During inspiration the chest expands and the stethograph is stretched, the pressure inside the stethograph decreases and is transmitted to the tambour and the atmospheric pressure

pushes the diaphragm, along with the lever, downwards. Down-stroke of the tracing thus represents inspiration and upstroke expiration.

During drinking of water, there is a temporary stoppage of respiration—this is called *apnoea* (deglutition apnoea). During talking, the movements become irregular.

During coughing, a deep inspiration is taken and is followed by a short expiratory blast, the air escaping through the mouth, while during sneezing the air passes out through the nose.

During breath-holding, a straight line is inscribed after which the respiratory movements are quicker and deeper.

While breathing into and out of the polythene bag, the subject breathes the same air over and over again. The amplitude and rate of the tracing increases.

After a bout of hyperventilation (hyperpnoea), there is a temporary pause in breathing after which the breathing returns to normal. After muscular exercise the respiratory movements recorded are deep and fast.

The time tracing helps in calculating the rate under normal and modified respiratory movements.

Discussion. The normal extreme limits of respiratory rate range from 12 to 24 per minute, most persons having a rate of about 15/min. The terms tachypnea and bradypnea refer to rates above and below these limits respectively.

Normal respiratory movements. The normal rhythmic respiration is regulated by neural and chemical means. Raised PCO_2 and H^+ , and decreased PO_2 stimulate respiration, the effects being mediated via respiratory chemoreceptors. These include carotid and aortic bodies, and a chemosensitive area located on the *ventral surface of medulla*. The medullary area is sensitive to cerebrospinal fluid H^+ ion concentration (CO_2 can easily pass through the blood-brain and blood-CSF barriers while H^+ and HCO_3^- penetrate very slowly). It is believed that, these 'medullary area

neurones' are stimulated by H^+ in CSF, and send impulses to the 'classical medullary respiratory centre'. Decrease in arterial PO_2 (hypoxia), reflexly stimulates respiration but its *direct* effect on the respiratory centre is depressant through and through. Pulmonary ventilation (and so, respiratory movements) is very critically adjusted by arterial PCO_2 . Increased PCO_2 is a 'stimulus par excellence' for the respiratory centre.

Modified acts of respiration. Deglutition apnoea is due to the stimulation of sensory nerve endings (9th nerve) in the pharyngeal wall, which reflexly causes contraction of adductor muscles of the vocal cords (10th nerve) resulting in closure of the glottis (the opening between the two vocal cords). This has a protective function in that it prevents aspiration of fluid or food into the lungs. Coughing is also a protective reflex, during which a deep inspiration is followed by interrupted expiratory blasts ; the force of expired air being strong enough to remove any mucous or irritating foreign material. Sneezing has the same pattern, except that the afferent impulses arise from the nasal mucosa and the air is forced out through the nose. Holding the breath (voluntary apnoea) for 30-40 seconds causes accumulation of CO_2 and a progressive fall in O_2 in the body resulting in increased rate and depth of breathing. During voluntary hyperventilation, the CO_2 is washed out and after it is discontinued, the respiration is depressed or it may stop altogether (apnoea). During this period, CO_2 accumulates and breathing starts again. This oscillation may be repeated 2-3 times but is quickly damped. Hyperventilation should not be carried out for prolonged periods, as the resulting alkalosis may precipitate an attack of tetany (due to fall in ionic calcium, resulting in an increased excitability of nerves). Psychological factors—anxiety, fear emotions are common causes of hyperventilation, but seldom produce alkalosis.

Rebreathing into the polythene bag results in stimulation of respiration. Experiments for demonstrating the CO_2 as a respiratory stimulant and breathing of gas mixtures must be carried out with caution and under the direct supervision of the teacher. During muscular exercise, increased production of CO_2 as well

as the increased demands for O_2 interact to stimulate respiration, besides other factors.

Precautions. (1) *The subject should avoid looking at the kymograph throughout the experiment.* (2) Voluntary hyperventilation should not be carried out for long. (3) The stethograph should be so adjusted that the movements of the lever are maximum.

Questions : (1) On the graph, which is inspiration-down-stroke or upstroke-and why ? (2) Describe the process of expansion of lungs during inspiration. (3) How is normal rhythmic respiration maintained ? (4) Name the muscles of inspiration and expiration. Is expiration an active process ? (5) What is deglutition apnoea ? What is its significance ? (6) Trace its reflex arc. (6) Name some other causes of apnoea. (7) Trace the reflex pathways of coughing and sneezing. (8) What are the effects of a bout of hyperventilation on respiration ? (9) What are the factors that increase ventilation during exercise ? (10) What is the composition of inspired, alveolar, and expired airs ? (11) When you rebreathe in a polythene bag, what happens to your respiration and why ? (12) Is CO_2 purely a waste product or does it serve any useful purpose in the body ? (13) What is the normal PH of blood ? what is acidosis and alkalosis ? What is 'alkali reserve' and what is its function ? (14) What are the concentrations and partial pressures of O_2 and CO_2 in the arterial and venous blood ? (15) What are the indications for artificial respiration ? Which is the method of choice in most cases ?

Experiment No. : 6.4
BREATH HOLDING TIME (BHT)

Breathing can be voluntarily stopped for a variable period by different individuals depending upon the status of lungs, deve-

lopment of respiratory muscles and practice. Age and sex affect the BHT.

Procedure. As the students work in groups of two, one becomes the *subject*, and other acts as an *observer*. The subject should sit quietly for 2-3 minutes, breathing normally before the breath-holding exercises are started. The observer uses a stopwatch and records the time for each determination. Each student should make three observations and note and tabulate these results in the note book.

Pinch your nostrils with thumb and index finger and hold your breath after a normal inspiration ; the observer notes the time for which breath can be held. Repeat the procedure twice at 2-minute intervals.

Record the BHT, using the same technique, after—(i) normal expiration, (ii) deep inspiration, (iii) deep expiration, (iv) breathing deeply and quickly for 20 times, (v) rebreathing from a polythene bag for 15 seconds, with a nose clip on, and breathing pure oxygen from a Benedict-Roth apparatus for 2 minutes.

Discussion. Measurement of breath-holding time is an indication of ventilatory drive as it is assumed that in a normal subject, the maximum voluntary inhibition that can be exerted over the ventilatory drive remains constant. The *breaking point* is reached when the ventilatory drive (due to high PCO₂ and low PO₂) exceeds the maximum voluntary inhibition of respiration. The atmospheric air contains 21 per cent oxygen and 0.04 per cent CO₂. During breath holding, oxygen is being continuously used up by the tissues along with accumulation of carbon dioxide, resulting in a state of asphyxia (hypoxia+hypercarbia).

Breath holding time is greater after hyperventilation (CO₂ washed out) and after breathing pure oxygen. It is least when a subject rebreathes from a polythene bag. After breathing exercises and practice, the BHT can be increased by 20-40 seconds. Normal breath holding time after a deep inspiration is about 50-70 seconds. Any value below 30 seconds requires

further investigations of pulmonary functions. BHT is decreased in many lung diseases e.g., in chronic bronchitis with emphysema, pulmonary oedema, tuberculosis and asthma. The purpose of taking three readings is to familiarise the subject with the procedure ; the third reading being the maximum in most subjects. While giving a report on BHT in a patient, it is essential to mention whether it was determined after a normal inspiration or a deep inspiration. The only precaution to be taken is to ensure that no air is inspired while the nostrils and mouth are closed.

Questions : (1) What changes occur in the body when breath is held for some time ? Does this situation resemble asphyxia ? (2) Explain the physiological basis of 'breaking point'. (3) Can breath holding time be prolonged by any means ?

Experiment No. 6.5

LUNG VOLUMES, CAPACITIES AND NOMENCLATURE, SPIROMETRY

The following nomenclature in respiratory physiology regarding the subdivisions of total lung capacity is generally accepted. The term "volume" indicates a fraction of subdivision of the total lung capacity ; "capacity" is used where two or more volumes are included in a measurement, for example vital capacity includes expiratory reserve volume and inspiratory capacity. Fig. 6.2 shows various lung volumes and capacities.

1. **Tidal Volume (TV).** Volume of air which passes in or out of the lungs during ordinary quiet respiration (400-500 ml.).
2. **Inspiratory Capacity (IC).** Maximal volume of air which can be inspired from the resting end-expiratory position ; it includes tidal volume (3000 ml.).

3. **Inspiratory Reserve Volume (IRV).** Maximal volume of air which can be inspired from the spontaneous end-inspiratory position ; it does not include tidal volume (2500 ml).

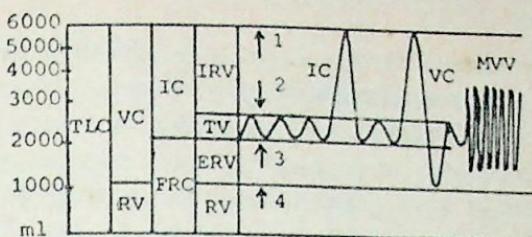


Fig. 6.2 : The lung volumes and capacities. The volumes do not overlap ; the capacities are made up of two or more volumes. Arrows :
1. Maximum inspiratory level ; 2. Resting inspiratory level ;
3. Resting expiratory level ; 4. Maximum expiratory level.

4. **Expiratory Reserve Volume (ERV).** Maximal volume of air that can be expired from the spontaneous end-expiratory position (1000 ml).

5. **Residual Volume (RV).** Volume of air remaining in the lungs at the end of a maximal expiration (1,000-1,500 ml).

6. **Functional Residual Capacity (FRC).** This is the volume of air remaining in the lungs at the end of resting expiratory position. It equals $ERV + RV$ (2,000-2,500 ml).

7. **Vital Capacity (VC) (Forced Vital Capacity—FVC).** Maximal volume of air that can be expired with a maximum effort after a deep inspiration (4,000-5,000 ml).

8. **Total Lung Capacity (TLC).** This is the volume of air in the lungs at the end of a maximal inspiration (6,000 ml).

9. **Minute Ventilation (MV)** (also called pulmonary ventilation ; respiratory minute volume). This is the volume of air breathed in or out in one minute during quiet respiration. Though it shows considerable variations in health, its relation to other measurements is of clinical importance (6-10 liters).

10. **Maximum Voluntary Ventilation (MVV).** This was formerly termed Maximum Breathing Capacity (MBC). It is

that volume of air which can be moved in or out of the lungs with maximum voluntary effort during one minute (100-140 liters).

Spirometry

A spirometer is used as a routine in physiological and clinical studies for the measurement of lung volumes and capacities. The instrument is a counterpoised gas holder. A writing point carrying ink, is fixed to the counterweight and inscribes on a variable speed electric kymograph cylinder with a graph paper wrapped around it. The respiratory movements are not affected by the spirometer as it offers very little resistance to the movement of air from the lungs of the subject. (The recording devices have low inertia and employ wide-bore corrugated tube airways and light-weight gas bells). Many varieties of spirometers are in use ; the record obtained with one type is shown in Fig. 6.2 along with the subdivisions of total lung capacity. A demonstration will be given by the teacher to show you its operation and the interpretation of the record obtained. The normal values vary with body size and age ; these must therefore, be predicted for each individual for comparison with the observed values. Furthermore, the relationships between various volumes and capacities provide valuable information in the diagnosis and prognosis of lung diseases. Some important relationships are given below :

A. Breathing reserve. MVV minus MV=100—10=90 liters.

B. Breathing reserve per cent or dyspnoea index.

$$\frac{\text{MVV} - \text{MV}}{\text{MVV}} \times 100$$

Normal values range from 90-92 per cent. When breathing reserve per cent is reduced to 60-70 per cent, it is called 'dyspnoea point', at which breathlessness or dyspnoea is present in the patient.

C. Ventilatory reserve. $\frac{\text{MVV}}{\text{MV}} = \frac{100}{10} = 10$ i.e., the pulmonary ventilation can be increased 10 times.

D. Ventilatory equivalent. This means as to how many liters of air have got to be ventilated for the absorption of 100 ml of oxygen. Average=2.75 liters. Extreme values=1.68—4.5 liters.

E. Air velocity index

$$\text{AVI} = \frac{\text{Per cent predicted MVV}}{\text{Per cent predicted VC}} \text{ Normal}=1.$$

F. Diffusing capacity of the lungs. This refers to the quantity of a gas that diffuses each minute for each mm Hg difference in the partial pressure of this gas across the respiratory membrane.

Normal : $O_2=20 \text{ ml/min/mm Hg}$ at rest

$CO_2=10-30 \text{ ml/min/mm Hg}$ at rest.

Questions : (1) What is meant by the terms 'capacity' and 'volume' in relation to the air of the lungs ? (2) Name the divisions of the air of the lungs. (3) How is maximum ventilation volume determined ? What is its significance ? (4) What is meant by the term ventilatory reserve ?

Experiment No. : 6.6

DETERMINATION OF FUNCTIONAL RESIDUAL CAPACITY

The method is simple and requires a large spirometer drum and a nitrogen meter. The subject breathes pure oxygen for 5 minutes, and the expired air is collected in the drum which has previously been washed out with oxygen so that it is nitrogen-free. The concentration of nitrogen in the expired air from the subject's lungs is estimated with the nitrogen meter. Since it is known that the alveolar air contains 80 per cent nitrogen (an alveolar sample can be collected and analysed), the FRC can be

calculated from the total volume of expired air and its nitrogen concentration. For example, if 40,000 ml of expired air in the spirometer contain 5 per cent nitrogen, then 2,000 ml of nitrogen must have been washed out of the lungs and as the lungs contain 80 per cent nitrogen, the FRC must be

$$2,000 \times \frac{100}{80} = 2,500 \text{ ml.}$$

The FRC increases as the subject assumes a sitting and then a standing posture ; this is due to the descent of diaphragm and abdominal contents under the effect of gravity.

When spirometry is combined with a determination of functional residual capacity, it is possible to calculate the total lung capacity (TLC) and residual volume (RV).

Experiment No. : 6.7

VITAL CAPACITY (FORCED VITAL CAPACITY : FVC) AND FORCED EXPIRATORY VOLUME: (FEV)

The electrically operated spirometer used for measuring vital capacity, other volumes and capacities, and "timed vital capacity" or the 1-second forced expiratory volume (FEV₁), is usually not available for student work in the general laboratory. Instead, the students estimate the vital capacity with a simple spirometer. This apparatus consists of an outer container filled with water in which a 6-liter capacity gas bell floats. The gas float is attached to a chain which passes over a calibrated pulley bearing an indicator needle (the needle moves with the pulley) which denotes the volume of expired air. The gas bell is counterpoised and has very little inertia and friction. The inlet tube is cor-

rugated rubber-canvas, bearing a metallic mouthpiece and is attached to a pipe fitted at the bottom of the spirometer (Fig. 6.3).

TABLE 6.1. Report on Vital Capacity

<i>Vital capacity</i>	<i>Ist reading</i>	<i>2nd reading</i>	<i>3rd reading</i>	<i>Maximum value</i>
1. Standing position
2. Sitting position
3. Supine position
For Report : Maximum value=.....				

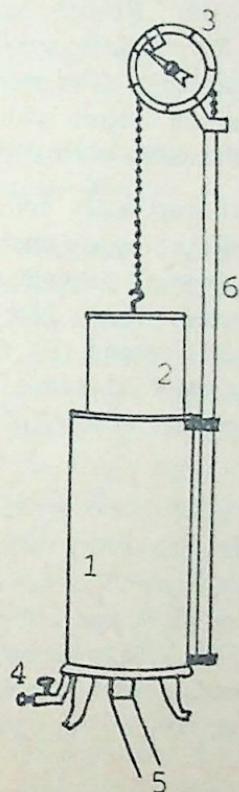


Fig. 6.3 : Simple spirometer. 1. Outer container ; 2. Gas float ; 3. Calibrated pulley with spring mounted indicator needle ; 4. Tap for draining water ; 5, Wide-bore inlet tube. 6. Counterpoise system.

Procedure. (1) Bring the gas bell to its lowest position so that the pointer at the calibrated pulley indicates zero i.e., the spirometer is empty, (2) Breathe normally for 6-8 times. Then, inspire as deeply and fully as possible, and while *keeping the nostrils closed* with thumb and index finger, expire into the mouthpiece (which is held tightly between the lips) with a maximum effort. The gas bell moves up and the pointer on the pulley indicates the volume of expired air. The forced expiration should be deep and quick, but without undue haste. (3) Repeat the procedure twice at 2-minute intervals. (4) Record the vital capacity in standing, sitting and supine positions to note the effect of posture.

Observations and results. Record your observations as indicated in Table 6.1. *Report* the maximal value in the standing position as the vital capacity. The purpose of taking three readings is to familiarise the subject with the procedure; the third reading being the maximum in most subjects.

Forced expiratory and inspiratory spirograms are recorded in many ill and weak patients who are unable to perform such vigorous tests as MVV. In such patients the '1-second forced expired volume' (FEV₁) test is done. Normally, 80 per cent of the air is expired in the first second, 93 per cent in the 2nd second and 97 per cent in the third second (the 3-second "timed" vital capacity). The important ratio—FEV₁/VC—called FEV % can be easily calculated.

Discussion. It is quite easy to determine most of the ventilatory values, but there are many variables which must be considered before giving a report. It has to be decided whether the values obtained for a particular individual lie within the limits of normal range. Prediction formulae have been derived to express correlations between vital capacity (and other respiratory quantities) and height, weight, body surface area and sex of the individual.

Factors affecting vital capacity and prediction formulae. 1. Relation to height=Height in cm \times 25 (males); \times 20 (females); \times 29 (athletes) e.g., with a height of 160 cm in a male, the predicted VC would be $=160 \times 25 = 4000$ ml.

2. Relation to BSA. Males = 2.5 l/m^2 BSA, Females. = 2.1 l/m^2 BSA (body surface area).

3. Relation to body weight. For an average healthy person the prediction formula is— $VC (\text{in ml}) = W^{0.72}/0.690$, where W is the weight in grams.

A formula which takes age also into account, both for males and females, is :

$$\text{Males: } [27.63 - (0.112 \times \text{Age})] \times \text{Height in cm}$$

$$\text{Females: } [21.78 - (0.101 \times \text{Age})] \times \text{Height in cm}$$

4. Occupation of the individual. Vital capacity also varies with the type of work an individual does and the use to which he has put his respiratory apparatus. Athletes, swimmers, divers etc. have a higher vital capacity. It is also increased by practice. The VC is low in subjects with sedentary habits.

Variations in vital capacity under pathological conditions. VC is low in the following conditions :

(a) Mechanical interference with the enlargement of the thoracic cavity—(i) abdominal conditions—tumours, pregnancy, ascites, flatus and abdominal pain, (ii) pleuritic pain, deformities of spinal column, ribs and sternum. (b) Conditions involving the lungs directly—pneumonia, tuberculosis and emphysema. (c) Space occupying lesions (these encroach upon the space normally occupied by the lungs e.g., pericardial and pleuritic effusions). (d) Depression of respiratory centre by narcotics, injuries to cervical spinal cord, poliomyelitis and myesthenia gravis. (e) Obstruction of respiratory passage (larynx, trachea, bronchi and bronchioles). (f) Pulmonary edema due to any cause. (g) Exophthalmic goitre (VC is low, presumably due to muscle weakness).

Effect of posture. Vital capacity is maximum in the standing position. In sitting and supine postures, there is muscular hindrance to maximum expansion of lungs (in supine position, the abdominal viscera push up the diaphragm and hinder its descent during inspiration).

Timed vital capacity. From the above discussion it is obvious that vital capacity is reduced most commonly when normal expansion of the lungs or the chest wall is decreased due to any reason. Vital capacity and timed vital capacity are still the simplest and most valuable tests for assessing lung functions.

Figure 6.4. shows the expiratory spirogram tracings in normal subjects, and in patients with *obstructive* and *restrictive* lung diseases. Normally, when measuring vital capacity, a person takes about 4 to 5 seconds to fill his lungs fully and then forces out the expiratory volume in just over 3 seconds. The normal FEV₁ should exceed 70 per cent (i.e., FEV₁ should be more than about 80 per cent). In *airway obstruction* it is greatly reduced. The common causes of obstruction are asthma and bronchitis (these two conditions are partly reversible with bronchodilators), and emphysema (this is non-reversible). In pulmonary *restriction*, the common causes of which are abnormalities of the thoracic cage (e.g., kyphoscoliosis) and pulmonary fibrosis (here lung volume is reduced), the FEV % is normal (the shape of the recording is also similar to normal) but the vital capacity is reduced. In restrictive disease the peak expiratory flow rate is also normal (see experiment 6.9).

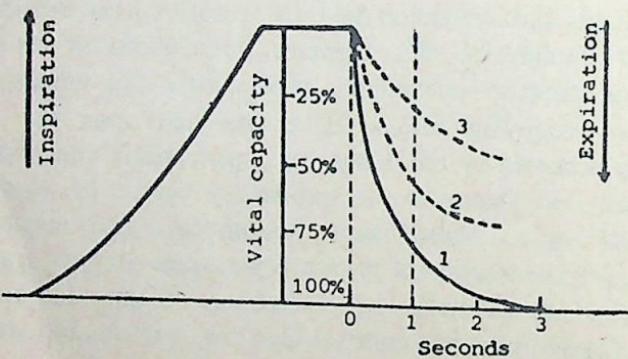


Fig. 6.4 The expiratory spirogram 1—normal ; 2—airways obstruction
3—restriction.

Precautions. (1) The subject must be made to understand what exactly he is expected to do during the test. (2) The observer should see that no air escapes through the nose or the mouth, and that the test is correctly carried out.

Questions. (1) Define vital capacity. What instructions will you give to the subject before recording it? How many readings are taken and why? (2) What are the normal values of vital capacity in males and in females? Can vital capacity be predicted in a subject? Which lung function is tested by vital capacity?

(3) How does posture affect vital capacity? In which posture is it maximum and minimum?

(4) Name the various physiological and pathological conditions that affect vital capacity.

(5) Can vital capacity be 'timed'? What are the normal FEV values? What special information may be obtained from FEV%? Name some other tests that assess (a) ventilatory, and (b) gas exchange functions of the lungs.

(6) What are 40 mm Hg and expiratory blast tests?
(See 6.13).

Experiment No. : 6.8

MAXIMUM VENTILATION VOLUME (MVV)
(SYN: MAXIMUM BREATHING CAPACITY)

The capacity of a subject to ventilate the maximum volume of air is determined and compared with the normal values. The expired air may be collected in a large spirometer drum (150 liter capacity) or a Douglas bag and its volume measured later with a gasometer. The MVV can also be determined with the direct-recording spirometer employed for measuring other lung volumes and capacities.

The subject is asked to breathe deeply and quickly for 15 seconds, in the standing position, and the expired air is collected in a Douglas bag. The volume of air is measured by connecting the bag to a gasometer and folding and refolding it tightly and repeatedly, so that no air remains in it. The air passes through the gasometer which indicates the volume directly.

Normal range=100-140 liters per minute.

Discussion. This index of ventilatory function requires complete cooperation of the subject and it is essential that he understands the procedure fully. Greater stress should be laid on the rate of breathing than its depth which should be subordinate to the rate, because more work is expended near the end of a deep expiration and inspiration as compared to the amount of air moved. The test is done for 15 seconds and MVV calculated for one minute, as it is difficult for a subject to continue this vigorous test for a full minute.

MVV is an important pulmonary function test as it measures defects both in *stroke volume* and *resistance*, whereas vital capacity a such, measures the volume defects only. These have been correlated and called capacity ratio. It is decreased in all conditions where vital capacity is decreased. The formulae for predicting the MVV are :

$$\text{Males: } [86.5 - (0.522 \times \text{age})] \times \text{sq m BSA}$$

$$\text{Females: } [71.3 - (0.474 \times \text{age})] \times \text{sq m BSA}$$

Consult Fig. 6.2. for lung volumes and capacities and a demonstration spirogram obtained in a normal subject.

Experiment No. 6.9

PEAK FLOW RATE-PFR

The Wright peak flow meter (Fig. 6.5) introduced in 1959, is a simple portable device for the measurement of ventilatory function. It measures the maximum flow rate or peak flow in a single forced expiration (it does not measure the volume expired, as spiroometers do). It has been extensively used in

mass surveys of lung function in general hospitals, chest clinics, and industrial health centres.

The apparatus can be used to distinguish reversible from irreversible lung disorders (asthma, emphysema, chronic bronchitis etc.) by measuring peak expiratory flow rate (PEFR) before and after inhalation of bronchodilator drugs. Thus, the response to treatment in bronchial asthma can be evaluated. The measurement of the effect of training in athletes is yet another application.

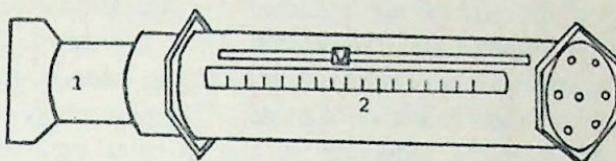


Fig. 6.5 A Wright peak flow meter. It directly measures expiratory flow rate. 1—mouth-piece ; 2—calibrated scale with marker. The calibrations are from 60 to 800 litres per minute.

The subject takes a deep breath and blows hard into the mouthpiece with a sharp blast, and the movement of the indicator needle on the dial is noted (there is, evidently, no need to empty out the lungs). Take six readings at 1 minute intervals, and record the average of four highest readings. Bring the needle back to zero by pressing the button located near the mouthpiece. Normal range = 350—500 liters/minute.

Questions. (1) What does a peak flow meter measure ? Does it measure the amount of air expired in one forceful expiration or does it measure the maximum flow rate in a single forced expiration ? (2) What is the normal range of PEFR in males and in females ? (3) What specific use can be made of this small and handy instrument ?

Experiment No. 6.10

GAS SAMPLING AND ANALYSIS

(A) Respiratory Gas Analysis

Haldane's respiratory gas analysis apparatus and its modifications have been in use for a long time, the basic principle in all being the same. Samples of inspired (atmospheric), mixed expired (from a Douglas bag) and alveolar air (last part of the expired air collected by the Haldane-Priestly method) are collected and transferred to a calibrated burette. The initial volume is measured and the sample exposed to KOH solution which absorbs carbon dioxide. The percentage of CO_2 is calculated from the reduction in the original volume. The gas sample is then exposed to pyrogallol solution, which further reduces the volume by absorbing oxygen. The gas remaining after the absorption of CO_2 and O_2 is nitrogen.

Medical gas analysers are now available where gas absorbing solutions are entirely dispensed with, and which respond rapidly and accurately to provide specific and continuous measurement of gases. These instruments are used extensively in pulmonary research, diagnosis of lung disorders and for monitoring patients under anaesthesia.

The composition and partial pressures of gases in the inspired, expired, and alveolar air is given in Table 6.6.

TABLE 6.6. Composition and Pressure of Inspired, Expired and Alveolar Gases

	Inspired air		Expired air		Alveolar air	
	Content	Partial pressure	Content	Partial pressure	Content	Partial pressure
Oxygen	20.84	159.0	15.3	116.5	13.2	101.0
Carbon dioxide	0.04	0.15	3.6	27.5	5.3	40.0
Nitrogen	78.62	597.0	74.9	569.0	75.3	572.0
Water	0.5	3.85	6.2	41.0	6.2	47.0
	100.00%	760.00 mm Hg	100.00%	760.0 mm Hg	100.0% 760.0 mm Hg	

(B) Blood Gas Analysis

The oxygen content and oxygen carrying capacity of blood is determined by Haldane's or Harris' blood gas analysis apparatus. Venous blood, collected under oil, is exposed to air in the apparatus so that the blood may pick up oxygen. All the oxygen is later expelled by potassium ferricyanide solution and its volume measured. The O₂ content and carrying capacity are then calculated as percentage. Modified Van Slyke volumetric method, using an electromagnetic apparatus, is employed for measuring the alkali reserve i.e., the carbon dioxide that can be liberated from the plasma sodium bicarbonate by the action of dilute sulphuric acid. (Approximately 60% of the CO₂ in the venous blood is in the form of bicarbonate).

TABLE 6.7. Normal Amounts and Partial Pressure of Blood Gases

Gas	Arterial blood			Venous blood		
	In simple solution	Total volume	Pressure	In simple solution	Total volume	Pressure
Oxygen	0.30%	19.0%	100.0 mm Hg	0.1%	14.0%	40.0 mm Hg
Carbon dioxide	2.40%	48.3%	40.0 mm Hg	2.7%	52.1%	46.0 mm Hg

Microgasometric methods are now available for measuring the oxygen and carbon dioxide content as well as their partial pressures in a given sample of blood. Table 6.7 shows the normal amount and partial pressures of O₂ and CO₂ in the blood.

*Experiment No. 6.11***EFFECT OF EXCESS OF CARBON DIOXIDE**

Work in groups of two, one student acting as the *Subject* and the other as *Operator*. Remove the soda lime tower from the Benedict-Roth apparatus so that CO₂ cannot be absorbed from the expired air. Fill the spirometer with 4-5 litres of oxygen, hold the rubber mouth-piece in position and close the nostrils with a noseclip. Adjust the tap of the spirometer and breathe room air for 2-3 minutes till the respiration becomes regular and then turn the tap so that breathing is to and from the spirometer. Record the respiratory movements for 4-5 minutes, but the experiment must be stopped if panting and discomfort tend to become too much. Observe the record obtained and discuss it with your teacher. In this experiment, there is no lack of oxygen, but, a gradual increase of carbon dioxide concentration occurs. The CO₂ acts on the respiratory centre, directly as well as reflexly, as explained before.

*Experiment No. 6.12***ARTIFICIAL RESPIRATION AND CARDIOPULMONARY RESUSCITATION**

Artificial respiration is indicated in two types of respiratory failure.

I. Sudden stoppage of respiration. This is seen in drowning, carbon monoxide poisoning, electric shock, overdosage with narcotics and anaesthetics, hanging, and some clinical conditions. In this group, artificial respiration must be started promptly by the first instructed person available on the scene of accident.

II. Gradually progressing respiratory failure is seen in paralysis of respiratory muscles in poliomyelitis, diphtheria and ascending paralysis. In these cases there is usually sufficient warning of the impending respiratory failure, during which time, arrangements can be made to employ respirators.

Methods of Artificial Respiration

The older methods of artificial respiration relied upon compression of the thorax to a position of forced expiration, thus allowing lung inflation to occur passively during the elastic recoil of the chest wall to the position of expiratory rest. This caused a movement of reserve air and not tidal air. These methods included : Schafer's back pressure method, Sylvester's arm lift chest pressure method, Thomson's hip lift chest pressure method and Eve's rocking method.

However, these prone or supine position methods of artificial respiration are no longer employed. Mouth to mouth respiration (exhaled air ventilation) has proved to be superior to all manual methods in all age groups. Comparative studies have proved it to be the only technique capable of producing adequate ventilation. The method is not only simple but also works by expanding the lungs.

Mechanical devices such as Drinker's tank respirator, Sahlin's jacket model and Brag Paul pulsator or their modifications for inflating the lungs intermittently are employed when artificial respiration has to be continued for long periods.

Mouth-to-mouth respiration. It is essential to provide and maintain a clear airway for the procedure to be effective, and any foreign material present in the oropharynx must first be removed with fingers, e.g., in cases of drowning. In infants, blow gently into mouth and nose and watch the movement of chest (18—20 times/min in infants and children).

(1) Extend the head fully and maintain head tilt. (2) Hold the lower jaw between the thumb and fingers and lift it up vertically. (Proper extension of neck, and the elevation of jaw prevents obstruction of the airway by the flaccid tongue). Use the other thumb and index finger to clamp off the nostrils. (3) Place your mouth over the subject's mouth and exhale twice the

tidal volume, noting the expansion of the chest at the same time. When the chest moves, take your mouth off to take another breath ; the recoil of the chest of the victim will empty the lungs. Repeat every 3-5 seconds till spontaneous breathing returns, or the patient is shifted to the hospital.

Heimlich Manoeuvre. This manoeuvre can prove life-saving in cases when a person begins to choke on something in his upper respiratory passages. Choking should not be mistaken for an attack of myocardial infarction. A person who is choking is usually unable to call for help but can only gesture while a person having a heart attack can usually speak (and, of course, choking is likely to occur while one is eating).

An immediate action is indicated. Stand behind the victim. Place your clenched fist just below his epigastrium and grasp your fist with your other hand. Then give a quick thrust inwards and upwards (the force must be applied to the abdomen and not to the thorax). This action pushes the diaphragm suddenly upwards so that air from the lungs of the victim is expelled forcefully through the trachea, carrying the foreign object out of the respiratory tract. The manoeuvre should be repeated until the airway is finally cleared.

The other emergency procedure is to bend the victim's head forwards and downwards so that it is lower than his chest. Then give several blows with the heel of your hand on the victim's back between the scapulae till the offending object is expelled. The head must be lower than the chest otherwise you may push the obstructing object further down rather than upward.

If you happen to choke while you are alone, position yourself over a hardback chair or some other firm object and thrust your abdomen quickly and repeatedly against the chair until the airway is cleared.

In many cases of choking, the respiratory passage is not completely blocked so that if the person breathes quietly and without panic, enough air may reach the lungs to keep him alive for a few minutes until he is shifted to the hospital.

Cardio-Pulmonary Resuscitation

The results depend on the effectiveness of training in resusci-

tation techniques and an appreciation of the importance of *Time Factor*. The most effective approach would be to train the medical students in collaboration with the anaesthetists. Many lives have been saved by timely help provided by laymen in cases of drowning, monoxide poisoning, heart patients collapsing on the roadside, and other emergencies. A detailed discussion of resuscitation methods is beyond the scope of this book however, the basics are mentioned below :

Phase I includes emergency measures.

A. Establish an open airway and give mouth-to-mouth respiration to provide adequate pulmonary ventilation and oxygenation.

B. If the carotid pulse cannot be felt and the pupils are dilated, start external cardiac massage by depressing the lower end of sternum by 4-5 cm, with one hand placed over the other at right angles. (a) One operator—alternate 1 quick lung inflation with 15 sternal compressions ; (b) Two operators—interpose one inflation after every fifth sternal compression. *Continue Resuscitation* until spontaneous breathing and pulse return.

Phase II is definitive therapy, which includes the restoration of normal breathing and circulation by the use of drugs, intravenous fluids, hypothermia, defibrillation and respirators in the intensive care unit (ICU) of the hospital. Continuous ECG monitoring is essential for assessing and evaluating the results of the treatment.

Experiment No. : 6.13

PULMONARY FUNCTION TESTS

It must be stressed that the pulmonary function tests help the clinician to make a *physiological* rather than a *pathological* diagnosis. What this means is that the tests will indicate that there is, for example, some obstruction to air flow but not that the patient has a specific disease—say bronchial carcinoma. Furthermore, they are more likely to be abnormal if there is a diffuse disease process affecting the lung tissue rather than when there is only a localised lesion e.g., tuberculosis. The tests may be useful in the following circumstances :

- (1) To indicate the degree of a patient's disability.
- (2) To differentiate between the possible causes of a patient's dyspnoea.
- (3) To follow the progress of a disease and its response to treatment.
- (4) To assess the respiratory status before anaesthesia and surgery, especially thoracic surgery where some part of the lung has to be removed.
- (5) To assess the physical fitness for various jobs.

The commonly used tests assess the—(a) Ventilatory functions and (b) Gas exchange in the lungs. These tests measure the degree and cause of respiratory disability. The first group measures lung size (vital capacity) ; patency of airways (MV_V, FEV₁) and alveolar ventilation ; efficacy of gas exchange is measured by determining gas tensions and oxygen saturation of blood. A simple classification is given below and the tests already described in detail are grouped in various categories :

I. Simple tests :

- (1) Breath holding time.
- (2) Chest expansion.

(3) 40 mm Hg test. The rubber tube attached to the mercury reservoir is disconnected from the armlet. The subject takes a deep breath and exhales into it and raises the mercury column to 40 mm Hg level. The time for which the mercury can be kept at this level is noted. Normal=40-50 seconds.

(4) Expiratory blast test. The level to which the mercury can be raised with a single forced expiration, after a deep breath, is noted. Normal=55-100 mm Hg.

(5) Snider's test. A normal adult should be able to blow out a burning matchstick held at a distance of 12 inches.

II. Spirometry. The following values are determined. (1) Minute ventilation at rest. (2) Inspiratory reserve volume and inspiratory capacity. (3) Expiratory reserve volume. (4) Vital capacity and timed vital capacity. (5) Maximum voluntary ventilation. (6) Peak expiratory flow rate (PEFR).

III. (1) Determination of functional residual capacity. (2) Lung compliance. (3) Dead space air. (4) Intrapulmonary gas mixing (IPGM). If pure oxygen is breathed, the nitrogen in

the lung gases is progressively diluted and washed out of the lungs in the expired air. Thus the measurement of pulmonary nitrogen elimination has been used as an index of perfect IPGM. Pure oxygen is breathed for 7 minutes, after which an alveolar air sample is collected and its nitrogen concentration determined; 2.5 per cent is considered as the upper normal limit. The pulmonary nitrogen emptying is defective under conditions found in emphysema with relatively large residual volumes.

IV. Gaseous exchange in the lungs is examined by :

1. Determination of PCO_2 and PO_2 by electrode techniques.
2. Determination of oxygen saturation of blood using spectrophotometric techniques.
3. Respiratory gas analysis.

V. Special techniques. (1) Plain skiagram of chest for evidence of lung disease. (2) Segmental intubation and injection of radio-opaque materials before taking skiagrams of lungs. (3) Laryngoscopy and bronchoscopy.

Experiment No. 6.14

CLINICAL EXAMINATION OF THE RESPIRATORY SYSTEM

Correct interpretation of history of illness of the patient and its correlation with the symptoms and signs like, fever, cough, dyspnoea, expectoration, haemoptysis, pain, cyanosis and clubbing of fingers, form the basis of diagnosis. For a detailed description of clinical examination, the student should consult standard books. Only an outline of the systematic manner of examination is given below. The subject should be examined in good light, stripped to the waist and preferably in the sitting posture.

I. Inspection. (a) Form of the chest. (b) Movements of chest. (see 6. I.).

II. Palpation. (a) Confirm the position of trachea by feeling for it in the suprasternal notch with the index finger, in relation to the insertion of sternomastoid muscles. Trachea may be pulled or pushed to one or the other side. (b) Locate the apex beat. (c) Palpate the axillary, supraclavicular, and neck regions for the presence of lymph nodes. (d) Place your hands in firm contact on the back of the subject, fingers spread out and the thumbs just touching in the midline. Ask the subject to breathe deeply. The movements of the thumbs away from the midline indicate equal or unequal expansion on one or the other side. (e) Vocal fremitus. Place the palm of the hand flat on the chest and ask the subject to say "ek-do-teen" or "one-two-three" twice or thrice each time. The vibrations which the voice transmits to the chest wall and felt by the hand are called *vocal fremitus*. The nature of the vibrations depends on the type of tissue lying under the hand. Compare identical areas on the back and front of chest as well as in the axillary regions. Vocal fremitus is increased when there is consolidation of lung tissue. It is decreased in pleural effusion with collapse of lungs.

III. Percussion. The procedure is described in the "Clinical Examination of Cardiovascular system." Begin in front and give a light and direct tap on the most prominent part of each clavicle. Then percuss other corresponding areas on either side in the usual manner. Carry out the percussion on the front, the back, and in the axillary regions, *always comparing identical areas on the two sides*. (The normal degree of resonance varies from person to person). Note areas of increased and decreased resonance, and the sense of resistance experienced by the finger in contact with the chest wall.

IV. Auscultation. Many extraneous factors may affect good auscultation e.g., outside sounds, rubbing of chest-piece with hair and skin and shivering. The student must become familiar with the normal breath sounds only then he can recognise altered and abnormal sounds. Make the following observations at identical

points on the chest. (1) Character of breath sounds, (2) Character of vocal resonance and (3) Presence or absence of other sounds. Breath sounds may be diminished or absent merely due to defective transmission, as in obese subjects.

1. **Character of breath sounds.** Breath sounds are composed of two basic elements—vesicular and bronchial. These two varieties can be heard over certain locations in healthy adults and children.

(a) **Vesicular breath sounds.** Vesicular breath sounds are produced by the passage of air in and out of millions of alveoli in the normal lung tissue. The sounds are heard both during inspiration and expiration. Throughout inspiration, the sound is fairly intense, rustling in character, and of low pitch. There is no clear cut pause between the end of inspiratory sound and the beginning of expiratory sound (Fig. 6.8); further, the expiratory sound is heard only in the early part of expiration, as is clear from the diagram. Thus, an important feature of vesicular breath sounds is that the inspiratory sound is heard for at least twice as long as the expiratory sound. Vesicular breath sounds are heard all over the healthy chest, but most typically in the axillary and infrascapular regions.

(b) **Bronchial breath sounds.** These sounds are produced by the passage of air through the trachea and large bronchi. The sounds are clear, blowing or hollow in character. The inspiratory sound is harsh and becomes inaudible just before the end of inspiration as shown in Fig 6.8. The expiratory sound has the same character but is generally more intense and of higher pitch than the inspiratory sound; the sound is audible throughout expiration. The silent gap between the inspiratory and expiratory sounds is therefore characteristic.

Bronchial breathing is normally heard over the trachea where it is very intense. Bronchial breath sounds replace vesicular breath sounds when a part of the lung becomes consolidated, especially when located near the surface. The common conditions associated with bronchial breathing are tuberculosis, pneumonia, and carcinoma. The sounds from the bronchi are

transmitted to the surface of the chest wall, passing directly through the diseased lung tissue instead of passing through millions of alveoli, as they normally do.

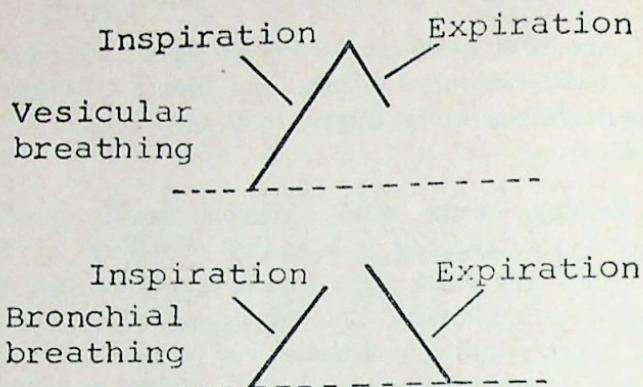


Fig. 6.8 Breath sounds.

To be able to recognise bronchial breathing, the student should place the chestpiece of the stethoscope over the trachea. This gives a fairly good impression of bronchial breathing that he will hear over diseased part of lungs. The essential difference is in the rhythm. Many variations of vesicular and bronchial sounds can be heard.

2. Character of vocal resonance. Note the intensity and character of the vocal resonance when the patient repeats "one two-three" or "ek-do-teen". Increased resonance is present in consolidation of lung tissue, e.g., lobar pneumonia.

3. Added sounds or adventitious sounds. (a) Rhonchi (dry sounds)—these are continuous 'snoring' sounds, when there is partial bronchial obstruction. These sounds are commonly heard in bronchial asthma.

(b) Crepitations (moist sounds)—these are discontinuous 'bubbling' or 'crackling' sounds and indicate the presence of fluid in small air passages and/or alveoli. Crepitations may be fine, medium or coarse.

(c) Pleural rub or friction, has a rubbing or creaking character, as if two pieces of leather were being rubbed together. It is heard in dry pleurisy.

Questions : (1) What is the normal shape of the chest ? What is Hutchinson's index ? (2) What is the normal rate of respiration ? What is the pulse respiration ratio ? (3) What types of respiratory movements are seen in children, females and males ? (4) How would you test for vocal fremitus ? What does it signify ? (5) What is the level of lower borders of lungs ? (6) What are the features of (a) Vesicular, and (b) bronchial breath sounds ? Can you hear bronchial sounds normally ? If so, in which region ?

Basal Metabolic Rate and Body Temperature

INTRODUCTION

The purpose of the ingested food is to provide energy for various body functions. The energy obtained by oxidation of foodstuff depends on the type of material being utilized. The unit of heat is the kilocalorie (kcal). One kcal (calorie or large calorie) is the amount of heat required to raise the temperature of 1 kg of water from 15°C to 16°C . The values for the energy obtained from the complete combustion of various materials are: Carbohydrates—4.1 kcal per g; Fats—9.3 kcal per g; and Proteins—4.1 kcal per g.

RESPIRATORY EXCHANGE RATIO

(RESPIRATORY QUOTIENT—RQ)

This is the ratio of CO_2 output to O_2 consumed—the respiratory exchange ratio i.e., $R = \frac{\text{Rate of } \text{CO}_2 \text{ output}}{\text{Rate of } \text{O}_2 \text{ uptake}}$ Usually 5ml of

oxygen is picked up by the tissues from every 100 ml of blood and 4 ml carbon dioxide is given out. The value of RQ changes under various metabolic conditions. A subject using an exclusively carbohydrate diet for body metabolism, will have an RQ

of 1.0. On purely fatty diet the RQ is 0.7 and in a person on mixed diet, the average value for the RQ is 0.825. The RQ has been extensively studied both in health and disease. It should, however, be noted that it does not provide any information about the metabolic processes in the body. For the interpretation of RQ, a knowledge of the metabolic processes taking place in the body is generally necessary. Furthermore, RQ is of no help in the study of the stage of intermediary metabolism. The effect of combustion of foodstuffs, change in pulmonary ventilation, violent exercise, and diseases like diabetes mellitus affect the respiratory quotient.

*Experiment No : 7.1***BASAL METABOLIC RATE (BMR)**

Principle. Basal metabolic rate is a determination of the amount of oxygen consumed by a relaxed and comfortable subject in the fasting state during a given interval.

Apparatus. (1) Benedict-Roth apparatus. It records the oxygen utilization through the recording of respiratory movements on a revolving chart paper. (2) Oxygen cylinder, and arrangement for measuring the height and weight of the subject. (3) Barometer and a thermometer.

Procedure. (1) Advise the subject to eat nothing after the evening meal and to go to sleep early. A sedative may be given at bedtime, the night before the test, if the patient is restless and cannot sleep. The subject should reach the laboratory in the fasting state and without any physical exertion. (2) Rest the subject for about an hour in comfortable surroundings. (3) Fill the bell of the apparatus with 4-5 litres of medical oxygen from the cylinder. Apply a nose-clip and insert a rubber mouth-

piece between the teeth and lips of the subject. Ensure that there is no leakage of air through the nose or mouth. Keep the tap of the apparatus at the 'air' position and connect the mouthpiece to the apparatus. Allow the subject to breathe room air for about a minute to familiarize him with the procedure and to allay any apprehensions before the actual test. (4) When the respirations become steady, switch on the apparatus and turn the tap to the 'test' position. The subject is now breathing pure oxygen from the bell, while the CO₂ in the expired air is being absorbed in the soda lime tower. The bell moves up and down with each respiratory cycle, and as more and more O₂ is consumed, the level of the writing pen continues to move up on the chart paper. Determine the oxygen consumption for 6 minutes, at the end of which, turn the tap to 'air' and remove the nose-clip and the mouthpiece. (5) Record the subject's height and weight, the room temperature, and the barometric pressure.

Observation and results. The subject's name, age, sex, height and weight are recorded on the BMR chart, along with the barometric pressure and room temperature. Blood pressure and the pulse rate are also recorded.

The rate of oxygen consumption is found by laying a foot-ruler along the lower turning points, corresponding to the beginnings of inspirations on the graph obtained. Calculate the BMR from the tables (nomograms) provided for the calculation of body surface area, and corrections for pressure, temperature, age and sex.

The normal BMR in men is about 40 calories and in the women about 35 calories per square metre body surface area per hour. The BMR is expressed as percent of normal. The normal variation is ± 15 per cent.

Discussion : It is essential that the subject should be fully relaxed and passive, and pay no attention whatsoever to the proceedings (the subject is asked to close the eyes and relax.) However, the person carrying out the test should be fully attentive throughout the test—thus ensuring that the apparatus is working correctly and that there is no discomfort to the patient.

The method described above is the "closed-circuit" method. In the "open-circuit" method, the expired air is collected in a Douglas bag over a period of time. A sample of this air is analysed for oxygen and carbon dioxide concentration with Haldane's apparatus. This permits the respiratory exchange ratio to be measured and a more accurate estimate of the calorific value of the expired air to be made.

The metabolic rate is the rate of production of free energy in the body. It is assumed that the whole of this energy appears as heat, so it is expressed as kilocalories per hour. The indirect method uses oxygen consumption rate as an indicator of heat liberation. The BMR measurement gives information about the thyroid gland activity, but BMR, as determined above, is not ideal in nervous patients. In such cases, somnolent metabolic rate (SMR) measurement is done after injecting sodium pentothal intravenously. SMR values in *normal* subjects are 8-12 per cent less than BMR values. The metabolic rate is influenced by age, sex, body surface area, starvation, body temperature, external temperature and food ingestion.

Metabolic rate may be determined for class work, at any time of the day, preferably 2 hours after lunch. Special respirometers are available to measure metabolic rate during work and muscular exercise, for example, on a bicycle ergometer. This is a convenient apparatus where a large portion of the body musculature is used. Band-brake type of bicycle ergometer is commonly used to measure muscular efficiency.

The metabolic rate in different animal species shows wide variations. However, the one variable that has a good correlation is body surface area (BSA). Direct measurement of BSA requires a time consuming and cumbersome technique in man. BSA in humans can be calculated from the following formula :

$$\text{BSA} = 0.007184 \times W^{0.425} \times H^{0.725} \quad \text{where}$$

W =Body weight in kilograms, and H =Height in centimetres.'

The basal metabolic rate is raised in :

1. Hyperthyroidism.

2. Many extrathyroidal factors may increase the BMR in euthyroid subjects—such as food intake, drugs, fever due to infections, an excesss of metabolising cells as in polycythaemia, leukaemia, acromegaly, pheochromocytoma, and pregnancy.

3. Faults in the technique—such as, failure to achieve basal state, leakage of oxygen from the apparatus or through a perforated ear drum—give false high BMR values.

The basal metabolic rate is lowered in :

(1) Hypothyroidism. (2) Malnutrition. (3) Addison's disease. (4) Post operative or postinfective weakness. (5) Faults in technique e.g., use of exhausted soda lime—give false low values.

Precautions : (1) The subject must be under complete basal conditions. (2) The room temperature should be comfortable as cold will produce shivering and thus increase the metabolic rate. (3) The apparatus must be checked from time to time for any leakage of oxygen, and the soda lime should be changed or charged as required.

Questions : (1) Comment on the metabolic record (Benedict-Roth) provided. (2) What is meant by BRM ? What instructions will you give to the patient before estimating the BMR ? (3) What happens to the CO_2 expired by the subject during the test ? (4) Name the conditions in which BMR is increased and decreased. (5) Which endocrine gland's function is assessed by this test ? Name some other tests for assessing the functions of the concerned gland.

Experiment No : 7.2

BODY TEMPERATURE

Man is homeothermic. The 'core' temperature remains fairly constant despite wide variations in the temperature of the surroundings. The normal range of the body temperature is from 97°F to 99°F or 36°C to 37.5°C. It depends on a balance between *heat production* and *heat loss*. Sources of heat production are (a) basic metabolic processes of the tissues, (b) muscle contraction and (c) the specific dynamic action of food. Heat loss occurs through (a) conduction and radiation of heat (about 60 to 70 per cent), (b) evaporation of sweat (about 20 to 30 per cent), (c) heat loss through expired air (1-2 per cent), and (d) heat loss through defaecation and micturition (about 1 per cent). There is a diurnal variation (nycternal) in body temperature, of about 1°-1.5°F, the minimum being in the morning and the maximum occurring in the evening. The temperature of the shell or superficial tissues varies widely, depending upon the temperature of the environment.

Recording the body temperature : It may be recorded in the mouth under the tongue, in the axilla or in the rectum. Rectal temperature is most reliable as it is near the core temperature. The oral temperature is measured routinely and is fairly reliable, provided the thermometer is kept in place for several minutes. (While recording oral temperature hot or cold drinks taken previously can affect the temperature considerably.)

The clinical thermometers are kept in a wide bottle or jar of antiseptic solution and are washed before use. The glass stem is shaped to act as a convex lens which gives a magnified image of the mercury in the capillary bore of the thermometer. The image is visible from a particular angle; that is why the thermometer should be slowly rotated till the mercury column comes into view.

Conduct the following experiments : (1) Hold the thermometer by the end away from the bulb-side and shake the mercury down into the bulb. Place it under the tongue and

close your mouth. Take the thermometer out after 30 seconds and record the reading. Shake the mercury down and record your temperature again by keeping the thermometer in position for one minute. Repeat the procedure and take readings after $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3 minutes and so on until a constant reading is obtained. The results may be plotted. Assuming that the highest reading is the true body temperature, find out how long the thermometer must be kept in the mouth ? (2) Rinse your mouth thoroughly with cold water at 10°C and record the oral temperature immediately after. Repeat with water at 50°C . (3) To determine diurnal variations, record your oral temperature at least 10 to 12 times in 24 hours. Try to obtain four readings during the night. Tabulate your findings. (4) Record your body temperature before, during, and after, light, moderate and severe exercise for a known period (during exercise, axillary temperature may be recorded as it will not be possible to keep the mouth open for an adequate time ; axillary temperature is usually 1°F less than the oral temperature). Record the time interval after which the temperature returns back to the pre-exercise level.

Thermocouples, taped to the skin, are employed for recording skin temperature.

Questions : (1) Record your oral temperature before and after exercise, and after taking a hot and then after taking a cold drink. (2) Why does the body temperature increase after exercise ? (3) What are the normal sources of heat production and heat loss in the body ? What is meant by core temperature ? (4) What precaution? will you take while recording the temperature of a patient ?

Cardiovascular System

INTRODUCTION

The discovery that the blood travels round in circuit in the body was made by William Harvey, physician to King Charles I, in 1628. This, perhaps one of the greatest discoveries in the field of medical science, of which indeed it laid the foundation, was the result of simple experiments and patient observations leading to logical conclusion. This was followed by fundamental discoveries and observations by many workers on the structure and action of the heart, haemodynamics, and the fact that the heart continues to beat *in vitro* for long periods under optimal conditions (the heart is nerve-regulated and *not* nerve-operated). The properties of rhythmicity, refractoriness, all-or-none law, among others, are well-known. The heart is a pump, in fact two pumps, placed in series, whose sole function is to receive blood from the veins and pump it into the arteries. With each contraction, about 70 ml blood is pumped out from each ventricle separately—the stroke volume. The ventricle, however, does not empty out completely; about 50 ml blood remains in it at the end of ejection of the stroke volume; this amount is called the *end-systolic volume*. It is important in increasing the cardiac output during muscular exercise where an increased blood flow through the tissues is required. The term *cardiac output* used unqualified, refers to the amount of blood ejected by each

ventricle *separately* in one minute. Thus with a heart rate of 70/min and a stroke volume of 70 ml/beat, the cardiac output would be 4900 ml/min (70×70) (beating 10,000 times a day, the adult heart pumps out about 7000 litres of blood through 96,500 kilometres of blood vessels). A correlation exists between the body surface area and cardiac output ; the output per minute per m^2 BSA (the cardiac index) averages 3.2 liters.

Experiment No. 8.1

INSPECTION OF PRECORDIUM

The portion of the anterior chest wall lying in front of the heart is called precordium. Observe if any pulsation is visible in the area. Usually, a definite cardiac pulsation is seen over a small area in the lower-outer region.

1. Try to locate the lowermost and the outermost *point* in the region of cardiac pulsation—this point is the apex beat. Confirm its location by palpation. Place your hand over the precordium—the base of your hand lying over the base of the heart and the fingers pointing towards its apex. Using the tip of your index finger, note the position of apex beat and mark it with a felt-tip pen. The normal apex beat lies in the 5th intercostal space, nine to ten centimetres from the midsternal line. It is commonly shifted to one or the other side in diseases of the heart and lungs (pushed or pulled towards a particular direction). The apex beat is a valuable physical sign in clinical practice. To locate the fifth intercostal space, first locate the sternal angle (angle of Louis) ; this is the junction between manubrium sterni and the body of the sternum. The second costal cartilage articulates with the sternum at this level! The second

intercostal space is below the second rib. You can now locate the fifth intercostal space.

2. Observe if there is any bulging or depression in the precordium ; normally, there is none.

Experiment No. 8.2 HEART SOUNDS

A stethoscope that combines a bell-type chest piece with a diaphragm type is better than the diaphragm type alone, as both have their uses under certain conditions in clinical examination of the chest. Place the chest piece of the stethoscope over the region of apex beat (*mitral area*) and try to distinguish between the first and the second heart sounds. The interval between the second and the first sound is greater than the interval between the first and second sound, during successive cardiac cycles (Fig. 8.1) Listen to the sounds over the area just to the left of the lower end of sternum (*tricuspid area*) ; over the right second inter-

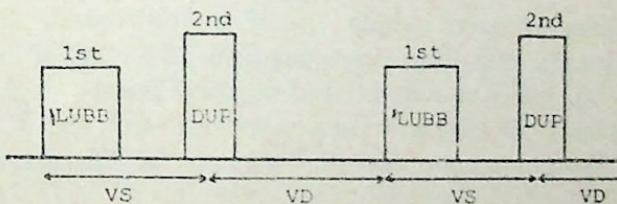


Fig. 8.1 : Diagrammatic representation of heart sounds. VS-ventricular systole, VD-ventricular diastole. LUBB and DUP-phonetic representation of 1st and 2nd heart sounds respectively.

costal space to the right of the sternum (*aortic area*) ; and over the left of the sternum in the second intercostal space (*pulmonary area*) (Fig. 8.2). These areas do not indicate the location of the

corresponding valves. The first sound is due to the simultaneous closure of atrioventricular valves and ventricular muscle contraction while the second sound is due to the closure of semilunar valves. Simultaneous palpation of carotid artery helps in identifying the first heart sound.

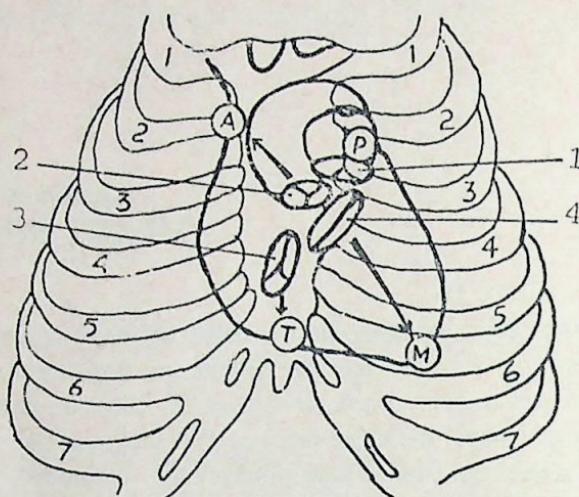


Fig. 8.2 : Diagram showing the projections of the heart valves and the auscultatory areas. 1—pulmonary artery valve ; P-pulmonary area : 2-aortic valve ; A-aortic area ; 3-tricuspid valve ; T-tricuspid area ; 4-mitral valve ; M-mitral area.

Features of heart sounds. (a) *First heart sound.* It is best heard over the apex beat, is prolonged (0.1-0.17 sec) of low pitch (25-45 Hz), and is usually the loudest sound heard over the heart. It is booming in character and coincides with the *R* wave of ECG. With high pulse rates, the intensity of first heart sound is increased.

(b) *Second heart sound.* It is abrupt and clear, has a short duration (0.1-0.14 sec), and of a higher pitch (40-50 Hz). It may precede, coincide, or follow the *T* wave of ECG. This sound may be split in some healthy subjects. It is best heard over the aortic and pulmonary areas. The intensity is commonly increased in hypertension.

Note : Tape-recorded heart sounds may be listened to before using the stethoscope, if possible. It will then be easier to appreciate the rhythm, general features and character of the normal heart sounds, as well as deviations from the normal in heart diseases. Phonocardiography reveals that a third sound and an atrial sound are generally present but difficult to hear with a stethoscope. When either of these are prominent and audible, they produce a triple rhythm (this may be represented phonetically as lubb—dup—dup) the third sound following the first two sounds. A tape demonstration will be given before this experiment.

Questions : (1) What is meant by the term precordium ? (2) What are the areas for auscultation of the heart sounds ? How are they related to the heart valves ? (3) How many heart sounds can be heard by the unaided ear and after recording ? How are they produced and what are their characteristic features ? (4) What is the relation of heart sounds to various mechanical and electrical events of the cardiac cycle ? (5) Name some abnormal sounds that may be heard over the precordium.

*Experiments No. : 8.3***EXAMINATION OF ARTERIAL PULSE**

The 'pulse' is the wave of increased pressure (pulse pressure) propagated centrifugally, with each ventricular ejection, at an increasing velocity (4-10 ml/sec), being amplified with distance within the major conducting arteries. The pulse does not occur synchronously in all arteries, nor is the extent of rise the same everywhere. It is important to distinguish between the transmission of pressure wave (the pulse) and the velocity of blood flow. (The velocity of blood in the aorta is

about 1 m/sec and in the capillaries about 1 mm/sec). Much information can be gained from the rate and quality of the pulse particularly in patients with disorders of the heart, but in this experiment, we are concerned with other parameters of the pulse as well.

Radial pulse is generally chosen, since it is easily accessible and lies against the bone. If it is aberrant, palpate the opposite radial artery. Whenever disease of the cardiovascular system is suspected, both radial pulses should be felt simultaneously and carefully compared.

Ask the subject to be seated comfortably and fully relaxed, with eyes closed. Note the presence of the main arterial pulses ; the common carotid, brachial, radial, femoral, popliteal, posterior tibial and dorsalis pedis (it is important in peripheral vascular disease), by palpating these vessels on both sides. Examine the radial pulse at the wrist with the tips of the first three fingers (index finger towards the heart), keeping the subject's forearm pronated and wrist slightly flexed. Note the rate, rhythm, volume, character, and condition of the vessel wall, in this order.

1. Rate : Count the number of beats for one minute. Make two more determinations at intervals of 5 minutes each, and record the three readings in your notebook. Compare the rate with the ventricular rate (counted by auscultation) and see if there is any pulse deficit. In atrial flutter and fibrillation, the rate at the wrist is less than the ventricular rate counted by auscultation at the apex. This difference is called the pulse deficit. It is due to the fact that some of the ventricular beats are so weak (due to shortened diastole, poor filling, and reduced stroke volume) that aortic valve fails to open so that no pressure wave is transmitted along the arteries.

(a) Normal pulse rate. The pulse rate is not constant in any one subject ; the pulse interval varying slightly from beat to beat. Thus the intervals between successive beats in a subject with a pulse rate of 60/min might vary between 0.7 and 1.3 seconds. Such fluctuations may be random, but there is a regular variation in pulse rate associated with respiratory

movements. The rate quickens during inspiration and slows down during expiration ; this is called *sinus arrhythmia* and is particularly noticeable in younger subjects.

Since examination of the pulse provides valuable information as to the state of the circulatory system and the general condition of the patient, the conditions under which the pulse rate is determined, must be recorded i.e., whether it was under basal conditions (early in the morning, before the subject has taken any meals or gets up out of the bed) or during the day time. Mention the conditions under which you have counted the rate—whether your work partner is nervous, or has lately hurried to reach the laboratory after meals. You have taken three readings not for the purpose of taking their average but to stress the point that the heart rate (and the pulse rate) is not constant during the experiment. Express your result as :

Pulse rate=.....per minute (Random reading)

Normal pulse rate=70-80/min Range=60-100/min

(b) Pulse rate above 100/min is called *tachycardia* and below 60/min *bradycardia*.

(i) Physiological tachycardia is seen in :

1. Emotional stress, nervousness, anxiety and apprehension (e.g., at the time of an interview) produce tachycardia.
2. In new-borns, the rate may be from 120 to 150/min. During infancy and early childhood, it gradually decreases.
3. The rate is comparatively high in females.
4. Diurnal variations : Comparatively higher rates are seen in the late evening and may reach above 100/min.
5. Muscular exercise.

(ii) Pathological tachycardia is seen in ;

1. Fever due to any cause—(the raised temperature acts directly on the SA node).
2. Atrial flutter and fibrillation.
3. Thyrotoxicosis.
4. In haemorrhagic shock, the pulse is fast and weak ('thready pulse').

(iii) Physiological bradycardia is present in—

1. Athletes. The resting pulse rate may be between 50 and 55/min, and is due to increased vagal tone.
2. During deep sleep and meditation.
3. The rate under basal conditions may be below 60/min.

(iv) Pathological bradycardia is seen in—

1. Myxedema.
2. *Heart block* : the rate depends on the degree of heart block. In complete (third degree) heart block the ventricular rate may be around 40 per minute (idioventricular rhythm.)
3. General weakness and debility following prolonged illness.

2. **Rhythm** : If the beats follow at regular intervals, the rhythm is regular. If the rhythm is irregular, try to assess the irregularity—whether it is occasionally irregular, with a pause following a beat (extrasystole), or the irregularity has a definite pattern (regularly irregular as in partial heart block, or irregularly irregular, as in atrial flutter and fibrillation).

3. **Character or form** : By character or form is meant whether the individual pulse wave has a normal rise, maintenance, and fall as the pulse is being palpated. Though the character of the pulse wave can be studied at the radial artery,

a better method is to palpate the carotid artery. Place your thumb on this artery at the level of thyroid cartilage and press backwards at the medial border of the sternomastoid muscle. Usually it is not possible to detect the waves of the normal pulse (see experiment 8.4) by palpation alone and slight variations from the normal may also be missed. However, in certain diseases an abnormality can be detected. Some of the common abnormalities are mentioned below.

(a) *Water-hammer, Corrigan or collapsing pulse.* The pulse wave rises and collapses rapidly. This is seen in aortic regurgitation and in some other conditions where there is leak from arterial system, as in patent ductus, and arteriovenous fistula. It is best appreciated when the patient's arm is elevated.

(b) *Anacrotic pulse.* The pulse wave has a slow upstroke, and is usually of small volume. This type is commonly seen in aortic stenosis,

(c) *Pulsus alternans.* Successive ventricular beats are, alternately, strong and weak. It indicates severe damage to the myocardium.

The forms of abnormal pulse waves described above can be seen on recording these with a sphygmometer.

4. **Volume** : Estimate, by gentle palpation, the amplitude of the movement or expansion of the artery during passage of the pulse wave. The volume is a rough guide to the pulse pressure, provided that the arterial wall is normal and not rigid. Pulse pressure depends on the stroke volume and compliance (expansibility) of the arteries. Thus, if the vessel wall is normal, the pulse volume gives an indication of the stroke volume. In older persons the vessels are more rigid (less compliant) so that the pulse pressure is widened for the same stroke volume. Record the pulse volume as small, medium, or large.

5. **Condition of the vessel wall** : Put enough pressure on the brachial artery with a thumb to abolish the pulsation in the radial artery, and then try to roll the empty artery against the underlying bone. In young subjects, the vessel wall cannot be

felt, or is soft. It is more easily palpable in older persons. In arteriosclerosis, it is felt as a thick, hard cord and may be tortuous. The same is true for other medium-sized arteries.

Another way to determine the condition of the vessel wall is to empty out the vessel by pressing hard on it with the proximal finger (the finger towards the heart), and palpating and trying to roll the vessel against the bone by the other two fingers.

6. Delay : Feel the left femoral artery and the right radial artery simultaneously. Normally the two pulse are synchronous. A delay in the femoral, compared with the right radial, is seen in coarctation of aorta.

Make the following observation in addition :—Feel the carotid pulse simultaneously with the radial pulse and try to determine whether they are synchronous. If not, which one occurs first, and why ? The pulse is felt slightly earlier in the carotid artery, this vessel being nearer the heart.

Description of a normal arterial pulse : The typical arterial pulse of a normal adult subject should be described in the following terms : the rate is 72/minute. The beats are regular in rhythm, normal in character, and equal in volume. The vessel wall is not palpable. The pulses are symmetrically present and there is no delay in the femoral pulse.

Note : While feeling (palpating) the pulse of a subject, slightly compress the radial artery against the underlying bone. The thumb is not used for palpating the pulse, because the pulse in thumb of many observers is sufficiently strong to be confused with the pulse of the subject. (You can test this in your own case by gently pressing your thumb in various positions against the surface of a table.)

Questions : (1) What is an arterial pulse ? What are the common sites where arterial pulse is examined ? (2) What features would you note while feeling the pulse ? (3) Name the physiological and pathological conditions in which the pulse rate is increased and decreased. (4) Name some causes of

disturbed rhythm. (5) What is meant by pulse deficit ? What is the mechanism of its causation ? (6) What is water-hammer pulse ? Name the condition in which it is encountered. (7) Draw a radial pulse tracing and label its components. (8) Compare the transmission of arterial pulse with the velocity of blood flow.

Experiment No. : 8.4

RECORDING THE ARTERIAL PULSE

Apparatus : Dudgeon's sphygmograph is employed for recording the arterial pulse. (*Sphgmo*=pulse). The padded knob is placed over the radial artery, the pulsations of which are transmitted to a finely balanced surface-writing hinged lever. A clockwork mechanism feeds smoked paper strips under the writing lever ; a time tracing can also be obtained.

These days, the radial pulse tracing is no longer recorded as a routine in clinical practice. An experienced physician can assess the cardiovascular status reasonably accurately by examining the pulse. Electronic recorders are available for the recording of pulse form and pressure when and if required.

Procedure : Mark the position of the radial artery at the wrist by a line draw with a felt-tip pen. Place the instrument on the wrist, with the padded knob on a point on the line where the pulsations are maximum. Keep the sphygmograph in the correct position with the straps and strings provided for the purpose. Adjust the pressure of the knob and the degree of flexion at the wrist which would yield a maximum excursion of the writing point. As the writing point moves with each pulse beat, depress the lever for moving the paper. Switch off the machine after 10-12 beats have been recorded and fix the graph in the usual way.

Discussion : The upstroke of the arterial pulse tracing (Fig. 8.3) is due to the expansion of the artery and the descending limb is due to its retraction. The steeply ascending limb is due to ventricular systole and corresponds to the maximum ejection phase (percussion wave). A small wave, the tidal wave, is seen in some records and is believed to be due to 'falling' of blood column over the aortic valve and leading to its closure. The most prominent wave on the descending limb is the dicrotic wave, followed by the dicrotic notch as shown in the figure. The dicrotic wave marks the end of ventricular systole and was believed to be due to the rebound of blood column from the closed aortic valve; however, it is more likely to be due to the elastic oscillations of the aorta at its natural frequency.

As the pulse moves down the arteries, its form changes due to friction, damping, and reflected waves from the periphery. These factors tend to increase the difficulty in interpreting the wave form. In aortic stenosis, the pulse wave rises slowly, and

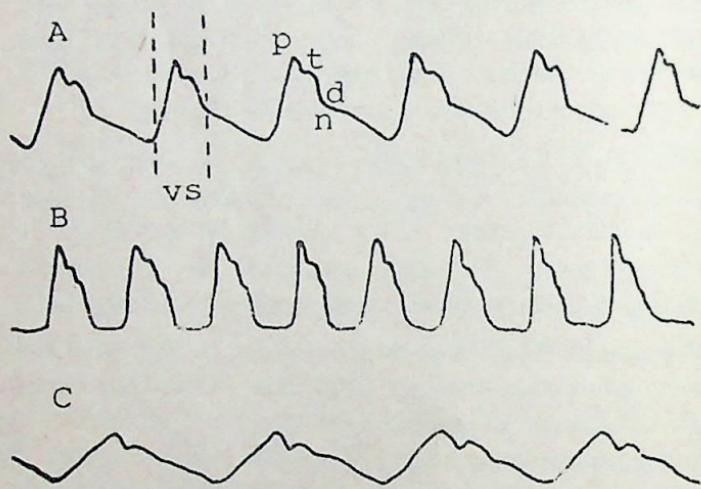


Fig. 8.3 : Forms of arterial pulse. (A) Normal pulse tracing ;—p—percussion wave ; t—tidal wave ; n—notch ; d—dicrotic wave ; VS—period of ventricular systole (aortic valve open) (B) Water—hammer (Corigan's ; collapsing) pulse showing rapid upstroke and descent. (C) Pulse tracing in aortic stenosis—showing a gradual upstroke and slow descent.

fades away leisurely (plateau pulse), while in aortic incompetence, the pulse rises sharply and falls quickly (collapsing pulse). Fig. 8.3. explains the different forms of pulse.

Experiment No. : 8.5

DEMONSTRATION OF CAROTID SINUS REFLEX

Method : Ask the subject to lie on the examination couch in the supine position (face up), and fully relaxed. Loosen the collar and lay bare the neck. Locate the anterior edge of the sternomastoid muscle, and feel the pulsation of the common carotid artery which lies deep and medial to the muscle. Locate the upper border of the thyroid cartilage ; the carotid artery and the carotid sinus are at this level. Compress the artery firmly with a thumb, against the vertebral bodies for *two seconds only*. The slowing of the pulse can be felt at this site as well as in a peripheral artery e.g., radial, which is palpated with the other hand.

Caution : *Do not compress both carotids simultaneously.*

Discussion : Compressing the artery stimulates the stretch receptors present in the tunica adventitia of the carotid sinus. Impulses travel along the carotid sinus nerve (the nerve of Hering), a distinct branch of the glossopharyngeal nerve. The response is a decrease in heart rate and fall of blood pressure. The nucleus ambiguus is the motor nucleus of the vagus, and the vagus nerve constitutes the efferent limb of the reflex are concerned with the slowing of the heart. The fall of blood pressure is due to the decrease in cardiac activity and the inhibition of tonic discharge in the vasoconstrictor nerves supplying the resistance vessels (arterioles).

Questions : (1) Indicate the site of location of the carotid sinus on your neck. What type of receptors are present in its wall and how are they stimulated ? (2) Describe the effect of rise of pressure and fall of pressure in the carotid sinus. (3) What is the effect of compressing (a) the common carotid artery below the carotid sinus, and (b) the carotid sinus, on the heart rate and blood pressure ?

Experiment No. : 8.6

RECORDING OF THE BLOOD PRESSURE IN MAN

The term 'blood pressure', used unqualified, refers to systemic arterial pressure. Its measurement in man is an important clinical procedure, as it provides valuable information about the circulation under normal and abnormal conditions.

A variety of factors like cardiac output, elasticity of blood vessels, vasomotor tone, viscosity of blood, and its volume operate to maintain the resting level of arterial pressure. The effects of factors like emotional stress, muscular exercise and posture, which produce transient changes in blood pressure, must, therefore, be excluded during its measurement.

Direct method : This involves puncturing and cannulation of an artery and was first employed in man by Faivre in 1856, for measuring the arterial pressure. These days accurate and continuous recordings of pressures in man can be obtained with strain-gauge, condenser or transducer manometers communicating directly with an artery through a needle. Such intra-arterial recordings are undertaken only in special circumstances, and are obviously not safe as a clinical procedure.

Indirect methods : Because of the inherent dangers of direct methods, indirect methods are employed which give reproducible results, provided necessary precautions are taken.

Definitions : Blood pressure is the lateral pressure exerted by the moving column of blood on the walls of the blood vessels. With the pumping action of the heart, the pressure rises to a maximum during systole and falls to a minimum level during diastole. The pressure in the arteries is thus pulsatile, and is due to the fact that the contents are more than the capacity at any given time.

Systolic pressure : This is the maximum pressure exerted on the walls of the vessels during systole.

Diastolic pressure : It is the minimum pressure in the vessels during the diastole of heart.

Pulse pressure : This is the difference between systolic and diastolic pressures.

Mean arterial pressure : It is the average pressure throughout a cardiac cycle. The duration of systole being less than that of diastole, the mean pressure is slightly less than the average of systolic plus diastolic pressures. A reasonable approximation is diastolic pressure plus one third of the pulse pressure.

Principle : In clinical practice, the blood pressure is measured with an instrument called sphygmomanometer. A cloth-covered rubber bag is wrapped around the upper arm and is inflated with air, till the higher extra-arterial pressure occludes the brachial artery. Pressure in the bag is then gradually reduced and balanced against the intraarterial pressure, and readings for systolic and diastolic pressures noted.

Sphygmomanometer. This word is derived from three Greek roots—'spymgo' meaning pulse, 'manos' means thin and 'meter' refers to measurement. In early procedures employing this method, the physicians used to feel the pulse and described its first appearance as 'thin', hence the term. The apparatus has the following component parts :

(a) *Mercury reservoir.* It contains mercury and is connected to the graduated tube at its lower end. A rubber tube attached to its opening at the top leads to an inflatable rubber bag. (b) *Graduated tube.* This is fitted in the lid of the apparatus along with the reservoir. It is graduated in millimeters (0-300), each division representing 2 mm (actually slightly less than 2 mm, since the diameter of the reservoir is not very much greater than that of the tube. When the mercury is driven up the tube for 20 mm, the meniscus in the reservoir falls, so that the actual pressure on the mercury in the reservoir is greater than 20 mm, and to compensate for this, the tube is calibrated with divisions which are slightly less than 2 mm apart). (c) *Armlet* (also called 'cuff'). A rubber bag 12 cm \times 24 cm, is enclosed in a strip of strong inelastic cloth material. This is wrapped round the upper arm where it keeps the rubber bag in position. Two tubes are attached to the bag, one transmits the air pressure to the mercury manometer, and the other is connected to the air pump. The width of the rubber bag should not be less than 12 cm for the average adult, 4 to 5 cm for children, and 2 to 3 cm for the newborn. With these dimensions, the pressure lost during the deformation of the tissues to occlude the artery, is usually below 6 to 8 mm Hg. (d) *Air pump.* It is a rubber hand bulb provided with a one-way valve at its free end, and a leak-valve arrangement at the other. A rubber tube connects the air pump to the rubber bag which can be inflated by turning the knurled screw clockwise, and alternately compressing and releasing the bulb. Deflation of the bag is achieved by turning the screw anticlockwise. The correct vertical position of the manometer is indicated by a click when the lid is fully opened.

Stethoscope. Although the stethoscope (*steth*=chest) was introduced by Laennec in 1819, it was not until 1905 that Korotkov used it in the measurement of blood pressure. It has two end pieces, the bell type and the diaphragm type (phonendoscope). In the latter, a plastic sheet is firmly held in position by a metal ring. The ear pieces are designed so as to conform to the direction of the external auditory meatus in each ear. The diaphragm type chest piece is employed for recording blood pressure.

Procedure. The subject may be either lying down or sitting, but must be physically relaxed, and free from excitement and anticipation. The upper arm should be at the level of the heart. Lay the arm bare upto the shoulder and ensure that no clothing is constricting the arm. The methods are :

1. **Palpatory method** (Riva-Rocci 1896). (1) Wrap the armlet around the upper arm, keeping its lower edge about 3 cm above the bend of the elbow. (The middle of the bag should lie over the brachial artery ; the rubber tubes will then lie over the anterolateral aspect of the antecubital fossa). (2) Locate the radial artery at the wrist and place three fingers over it. Hold the rubber bulb in your palm in such a way that your thumb and index finger are free to manipulate the leak-valve screw. Inflate the pneumatic bag and take the mercury to 40 to 50 mm above the level where the pulse disappears (this pressure is required to overcome the resiliency of the vascular wall and the soft tissues around it). (3) Reduce the pressure *gradually*. (5 mm at a time), thus bringing the mercury level down slowly. Note the reading when the radial pulse just reappears. The first appearance of the pulse is the **SYSTOLIC PRESSURE**. It corresponds to the time when the blood starts flowing through the compressed segment of the artery during each systole. It is easier to record the reappearance of pulse than its disappearance. The first 2 or 3 beats, being thin, may be missed, so that the actual systolic pressure is 6-8 mm higher than the recorded value. The disadvantage of this method is that the diastolic pressure cannot be determined. However, it is always employed before recording the pressure with the auscultatory method for reasons to be described later.

2. **Auscultatory method.** (Korotkoff, 1905). No sounds are ordinarily heard if the stethoscope is applied over the bifurcation of the brachial artery. If the cuff pressure is raised above the systolic pressure and then gradually lowered, a series of sounds are heard as the smooth laminar flow becomes turbulent and sets up vibrations. The cause of sounds is the small amounts of blood that are jetted at high velocity through the partially occluded artery, at the peak of each systole. These small quantities of blood strike against the stationary column in the distal

segment of the artery with a forceful impact. This impact causes turbulence and vibrations which are heard as sounds. Korotkoff described these sounds and correlated these with systolic and diastolic pressures.

The first sounds are clear, sharp, and tapping, but with a further reduction of pressure in the cuff, the character of the sounds changes (presumably due to changes in the degree of turbulence), a murmurish element being added to these. The sounds suddenly become muffled as the diastolic pressure is reached and disappear altogether when the blood flow becomes laminar once again (Fig. 8.4).

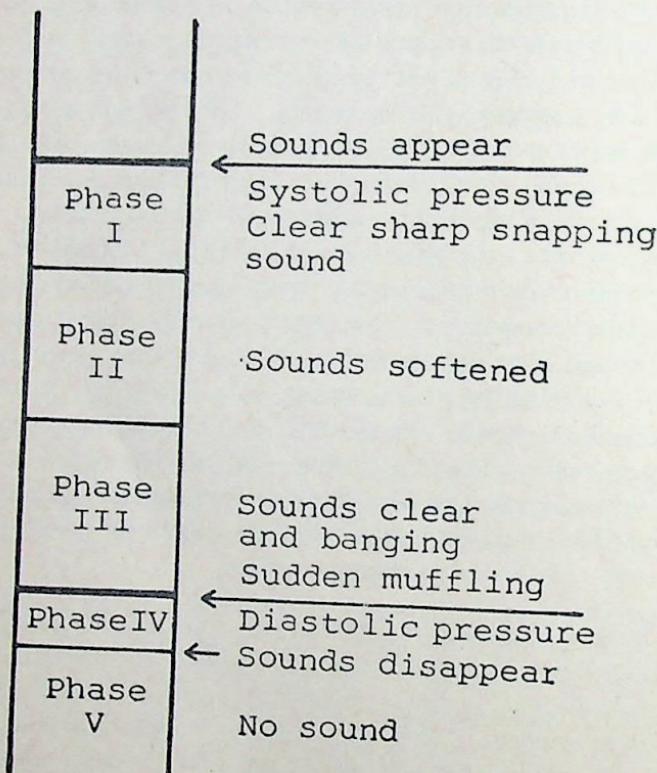


Fig.8.4 : Phases of Korotkov sounds heard during recording of blood pressure with auscultatory method. Systolic pressure—first appearance of sounds ; Diastolic pressure—sudden muffling of sounds.

It should be noted that all subjects do not show these series of sounds equally well. Indeed it may be difficult to identify any phases at all except the first appearance of sounds (the criterion for systolic pressure) and their sudden muffling (the criterion for diastolic pressure). In some subjects, especially in hypertensives, there may be an '*auscultatory gap*', in the sounds. As the mercury is lowered, the sounds may disappear for some time and reappear again as transition is made from phase I to phase III. If the mercury column is raised to this silent period only, one may miss the initial appearance of sounds, thus recording an erroneously low systolic pressure. The mistake can always be avoided by raising the pressure well above the systolic level as determined by the palpitory method. The palpitory method must, therefore, always precede the auscultatory method.

Procedure : (1) Locate the lower end of the brachial artery in the cubital space, just medial to the tendon of biceps. Place the diaphragm of the stethoscope over this region and keep it in position with the fingers and thumb of a hand, ensuring that it does not rub against the cuff or the tubes. (2) Inflate the cuff slowly and raise the pressure to about 30 mm above the systolic level as determined by the palpitory method. Lower the cuff pressure gradually until the first sound is heard, usually as a clear sharp tap. Record the reading at this point which marks the *systolic* pressure. Lower the mercury column further, at the same time listening to the sounds carefully. As deflation progresses the character of the sounds will be found to change, varying through murmurs to banging and loud, then suddenly becoming 'muffled' (dull and faint, as if coming from a distance) and finally disappearing. The manometer reading at the instant the sounds become muffled marks the *diastolic* pressure. Note the reading at which the sounds disappear completely, after which, deflate the bag quickly to zero pressure.

TABLE 8.1 : Record of Blood Pressure

Observer Self Subject	Pulse rate	Palpatory systolic	Auscultatory method		
			Systolic	Diastolic	Pulse pressure
Reading No. 1					
	2				
	3				
Average					

The following phases of the Korotkoff sounds can be differentiated (Fig. 8.4) :

Phase I. It begins with clear, sharp, snapping sounds. The appearance of the first sound marks the systolic pressure. The sounds become successively louder for the next 10 to 12 mm fall of pressure. *Phase II* : This is marked by a softening of the sounds which assume a murmurish character. This phase lasts for the next 15 mm. *Phase III* : Begins with the sounds becoming clear and 'banging.' The sounds increase in intensity for about 10 to 14 mm. *Phase IV* : This phase is signalled by a reduction in the intensity of sounds. The point at which the sounds become muffled is the accepted criterion of diastolic pressure and should be recorded as such. This phase lasts for about 5 mm fall of pressure. *Phase V* : This coincides with the cessation of all sounds.

The blood pressure is usually expressed as Systolic/Diastolic e.g., 120/80 mm Hg (new borns have a systolic pressure of between 20 and 60 mm Hg). Intra-arterial recordings of blood pressure have shown that the disappearance of sounds (phase V) corresponds more exactly with the diastolic blood pressure. Therefore, blood pressure may also be expressed as 120/80/75 mm Hg, the last figure indicating the disappearance of Korotkoff sounds.

While trained observers can record reproducible values, differences between readings obtained by two workers may vary

by as much as 10 mm Hg. The rate of deflation, acuity of hearing, concentration, and interpretation of sounds are the factors which give rise to these differences. A beginner may find difficulty in appreciating the muffling of the sounds, the true criterion of diastolic pressure. It is easy however, to note the disappearance of sounds. If 6 mm are added to this figure, the true level of diastolic can be arrived at. Practice is required to be able to record the pressure with any degree of accuracy. No opportunity should, therefore, be missed to familiarise oneself with the procedure of recording blood pressure.

Observations and Results : If the blood pressure is recorded without regard to mental and physical rest or ingestion of food, the fact must always be recorded, for example : 136/86 (casual reading).

- A. One student acts as the *observer* and the other, the *subject*. Record the blood pressure thrice at intervals of 5 to 7 minutes and note down the readings as shown in Table 8.1.
- B. Record the blood pressure in the sitting, standing and supine positions, to determine the effect of posture.
- C. Record the blood pressure in the other brachial artery as well as in both the femoral arteries.
- D. Record the blood pressure after muscular exercise of light and moderate intensity.
- E. Record the pressures with the arm raised above the head, and then, with the arm hanging down below the level of the heart.

Discussion Normal blood pressure : In most normal young adults the systolic and diastolic pressures average around 120 mm Hg and 76 mm Hg, the usual variations being 100-140 for systolic and 60-86 for diastolic. The upper normal limits for the two are usually considered to be 150 and 90 respectively. It should be remembered that variation within the normal is the rule. Ordinarily, there is no sharp demarcation line between normal and abnormal values, but *the farther away a reading lies from the average, the greater is the probability of the presence of disease.*

One often comes across a reading which lies near the limit of range for normals. What interpretation is to be placed upon such a reading? When only this isolated fact is available, one must always think in terms of probability. For example, if the BP is 150/90 there is greater probability of disease than if the reading were 130/84 which is near the average for normals. As a result one should carefully look for the cause of the elevated pressure. If there is no evidence of disease, then evidently, the pressure is normal for that individual. If the BP is 160/100 under resting conditions, there is even a greater probability that a disease process is causing the elevated pressure. If the pressure is *consistently* higher, then there is no doubt that the person is suffering from hypertension.

Systolic pressure: The stretching of the big elastic vessels buffers the pressure and does not allow it to rise to a high level. The systolic pressure represents the work done by the heart in overcoming the resistance of the vessels. The pressure falls only slightly in the large and medium arteries, because their resistance to blood flow is low. Maximum resistance is encountered in the *arterioles*. The pressure in the systemic arterioles falls from about 90 mm to 32 mm at the capillary ends. The systolic pressure shows greater variations under ordinary conditions of health as compared to diastolic.

Diastolic pressure. Clinically, greater significance is attached to this pressure, as the blood vessels are subjected to this pressure throughout the cardiac cycle, while the systolic level is reached only for a moment during each cycle. Diastolic pressure also reflects the state of peripheral vessels. Furthermore, since most of the coronary flow occurs during diastole, the diastolic pressure determines the filling of coronary arteries.

Pulse pressure. This results from the ejection of blood into the aorta and its magnitude, other things being equal, will vary with the quantity of blood ejected by the heart per beat. Increased peripheral resistance in the arterioles primarily increases the diastolic pressure (reduction in pulse pressure), whereas decreased resistance lowers it. The pulse pressure is very much increased in aortic regurgitation. Indeed it may be impossible to record

the diastolic pressure as the Korotkov sounds continue right up to zero. A slight pressure on the lower end of the brachial artery with a stethoscope alone may produce clear sharp and snapping sounds called pistol-shot sounds.

Blood pressure in the lower limbs. The subclavian and brachial arteries represent side arms from the wall of aorta. The pressure recorded in the brachial artery essentially represent the lateral pressure in the aorta. Much of the energy in the aorta is the kinetic energy or the energy of flow. In the lower limbs, the femoral arteries are the direct extensions of aorta. When these vessels are compressed, the flow of blood through them stops, and the energy of flow is thus converted into potential energy or energy of pressure. This pressure is, therefore, represented in the recording of blood pressure. For this reason, even in the supine position, the pressure recorded from the lower limb is somewhat higher than in the upper limb. A low pressure in the femoral artery with hypertension in the arms is the basic clue to the diagnosis of coarctation of aorta. With the normal subject standing, however, the femoral pressure is higher than in the brachial artery due to the pressure of the column of blood, due to the effect of gravity.

REGULATION OF BLOOD PRESSURE

The necessity of an adequate perfusion of the extensive capillary networks in the systemic vascular bed demands that the arterial pressure be maintained at sufficient levels. The blood pressure is, in a large measure, dependent on the cardiac output and the peripheral resistance. Cardiac output is controlled by (a) factors regulating the ventricular end-diastolic volume, (b) the myocardial contractility and (c) the heart rate. The autonomic nervous system has a major role in the maintenance of blood pressure by its influences on the cardiac output and on the extent of constriction of the arterioles (resistance vessels), venules and veins (capacitance vessels). Stretch receptors in the carotid sinus and the aortic arch constitute the afferent limbs of the autonomic reflex arcs which regulate the arterial pressure. Impulses from these receptors pass along the glossopharyngeal and vagus nerves (buffer nerves) to the medulla where they make

extensive connections. Connections are not only established with sympathetic and parasympathetic nuclei and efferent arcs, but also with cerebral cortex and hypothalamic nuclei, the latter controlling hormonal secretions from the posterior pituitary gland.

Activation of sympathetic outflow and inhibition of parasympathetic activity is produced by a rapid fall of blood pressure, which diminishes the excitation of the baroreceptors. The blood pressure is returned back to the control levels by vasoconstriction, increase in heart rate and force of contraction, secretion of adrenal medullary hormones, and release of ADH, ACTH and aldosterone. Opposite changes result from an acute rise of pressure. The operation of the baroreceptor mechanism, therefore, normally protects the body from a variety of influences, which would otherwise cause marked changes in blood pressure.

Variations in Blood Pressure under Physiological Conditions

1. **Age.** At birth, the systolic pressure averages about 40 mm, reaches 70 after 2 weeks and 80 at the end of first month. From 4-10 years systolic pressure averages 90-100 and diastolic 60-70. Adult levels are reached by 18-20 years and at 60 years it is 160/88.
2. **Sex.** The pressures are generally lower in females, but rise abruptly at menopause ; the pressure now remains a little above the male average.
3. **Body build.** Overweight persons have higher pressures, systolic as well as diastolic.
4. **Diurnal variations.** The difference in the morning and evening levels may be 10-12 mm Hg. The BP is lowest in the morning and the peak is seen in the late afternoon.
5. **Digestion :** The systolic pressure shows a rise of 6-8 mm Hg after meals and lasts for about one hour. Diastolic is little affected, if anything, it may decrease a little due to vasodilatation in the viscera.

6. **Emotions** : Anger, fear, worry, apprehension and excitement may markedly increase the systolic pressure. During sleep, it may fall by 10-30 mm. Diastolic is not much effected.

7. **Posture** : The blood pressure is least in the recumbent posture. Upon assuming erect posture, the systolic falls a little but soon returns to normal by the compensatory mechanisms.

8. **Exercise** : The pressure rises during exercise due to the operation of many factors.

9. **Relation to respiration** : The BP is elevated and lowered rhythmically, coinciding with respiratory movements. It generally starts rising during the latter part of inspiration and continues to rise in the early part of expiration, then it starts decreasing during the early part of inspiration. Species differences are often observed.

Variations in Blood Pressure under Pathological Conditions

Hypertension is classified into the following two groups :

I. **Essential hypertension** : In a vast majority of cases, no cause can be ascribed to the raised pressure.

II. **Secondary hypertension** : In about 10-15 per cent of all cases of hypertension, the cause can be found by a careful diagnostic approach. The discovery of the cause is important in that the cure of the condition is possible. This applies especially in children and young adults. The main causes of secondary hypertension are :

(a) *Renal diseases* : (i) Parenchymatous, (ii) Polycystic disease and (iii) Stenosis of renal artery. (b) Coarctation of aorta. (c) Endocrine diseases : (i) Pheochromocytoma, (ii) Primary aldosteronism (Conn's syndrome), (iii) Cushing's syndrome, (iv) Hyperthyroidism, (v) Hormone therapy (estrogens and oral contraceptives) and (d) Toxaemias of pregnancy.

Hypotension : Hypotension or low blood pressure is hardly, if ever, considered a disease or a cause of alarm in otherwise healthy subjects. There may be occasional and brief episodes of dizziness or faintness. Rarely, postural hypotension may occur

when a person gets up abruptly from supine position. Stability is, however, recovered in a few seconds.

Precautions : While recording the blood pressure, the following precautions are essential : (1) The pulse rate should be checked at the same time, as the BP is affected to some extent by the heart rate. (2) The cuff pressure should never be kept high for any length of time because reflex vasoconstriction occurring in peripheral vessels, would give falsely high readings. (3) Inflate the cuff quickly to a pressure about 30 mm above the level of systolic pressure as found by palpation and conduct the auscultation during slow deflation. (4) Sometimes the sounds are very indistinct ; in such cases the other arm should be tried. (Presence of gross oedema or contracted muscles can give unreliable readings).

Questions : (1) What precautions will you observe while recording the blood pressure ? (2) Define the terms-systolic, diastolic, mean arterial and pulse pressures. (3) Why is the apparatus called sphygmomanometer ? What is the principle on which the indirect method for recording blood pressure is based ? (4) Why must palpitory method be always employed before ausculatory method ? (5) What are Korotkoff sounds and what is the mechanism of their production. What are the criteria for systolic and for diastolic pressure ? (6) What is auscultatory gap and what is its significance ? (7) What is the range of normal blood pressure in adults and in children ? (8) How is blood pressure regulated ? (9) Name some physiological and pathological conditions in which the blood pressure may be raised. (10) What is hypertension ? How is it classified ?

Experiment No. : 8.7

**ELECTROCARDIOGRAPHY ECG
(SYN : EKG)**

ECG is a record of the electrical activity of the heart. Changes in electric potentials associated with the contraction of the heart can be recorded from different points on the surface of the body. The record obtained is called an electrocardiogram.

Apparatus (1) *Electrocardiograph* : The machine works on battery or electricity, and has a very sensitive galvanometer. The currents picked up from the surface of the body are electronically amplified before flowing through the galvanometer. The direct writing machine has either an ink-writing, or, an electrically-heated stylus, which inscribes on a chemically treated graph paper. The controls mounted on the apparatus are for calibration, centring the stylus, selecting the various leads, and for paper-speed control. A "patient connector cable" connects the electrode leads to the body surface. More sophisticated machines have arrangements for venous pulse tracing, phonocardiography, and for the continuous monitoring of ECG on an oscilloscope. When an ECG is to be taken, electrodes (flat metal plates) are placed on the skin and kept in position by rubber straps. Electrolytic jelly is rubbed on the area of the skin under the electrode to reduce skin resistance. The electrodes are then connected to the machine with 'cable leads', (2) *The graph paper*. The ECG graph paper is divided into one millimetre squares by thin lines. Every fifth line is thicker, both horizontally and vertically. Horizontally, one small square represents 0.04 second, so that the time duration between two thick lines is 0.2 second. Vertically, the amplitude of a wave is measured in millimetres. This facilitates quick calculations of the durations and amplitudes of various waves, and intervals of the ECG record.

Leads : The term 'lead' is employed both for the procedure of taking the tracing, and for the actual tracing obtained. When a person is connected to a machine, one electrode is always

applied to the right leg. This acts as an indifferent electrode and connects the subject to earth. There are two types of leads employed in practice.

I. Bipolar leads. (*Syn : Standard leads, Classical limb leads.*) The electrodes are appropriated marked for left arm, right arm, and left leg (left foot). The limb electrodes are fixed in position just above the wrists and on the front of the left leg just above the ankle.

(A) *Bipolar limb leads.* These were the earliest to be used. Both the electrodes record the electrical activity at their respective locations and represent the difference in potentials between the two limbs at three pairs of points.

Lead I : From right arm and left arm (RA—LA)

Lead II : From right arm and left leg (RA—LL)

Lead III : From left arm and left leg (LA—LL)

These leads are useful for most of the cases. By convention, negativity at the right hand electrode is recorded as an upward deflection.

(B) *Bipolar chest leads.* The exploring electrode is placed on the front of the chest in different locations and the other electrode on the right arm (CR 1-7); left arm (CL—1-7); and left leg (foot) (CF 1-7).

II Unipolar leads. These record the potentials from a single region of the body. One of the electrodes, *the indifferent electrode*, is kept at zero potential, by connecting the electrodes on right arm, left arm, and left leg, to a central terminal through a 5000 ohm resistance (this is achieved automatically in the machine itself). The currents from the three limbs neutralize each other, and there is no important potential change at the indifferent electrode during the cardiac cycle. The other electrode is the *exploring electrode* which can be placed on any area of the body. The ECG records the potential changes which affect the exploring electrode only.

(A) *Unipolar limb leads.* 'Augmented' limb leads are used these days. Thus we have aVR—right arm; aVL—left arm; and aVF—left foot (leg).

(B) *Unipolar chest leads* (also called unipolar percordial leads). These record the potentials from the anterior surface of the heart, from the right side towards the left side. The recordings are characteristic for each ventricle. These are labelled as VC 1—8 or V 1—8. Their positions are. V_1 =fourth intercostal space, right border of sternum; V_2 =fourth space, left of sternum; V_3 =between V_2 and V_4 ; V_4 =fifth intercostal space, midclavicular line; V_5 =fifth space, left anterior axillary line; V_6 =fifth space, midaxillary line; V_7 =fifth space, post-axillary line; V_8 =on infrascapular line, just below the angle of the scapula. The chest electrode is a small metal cup with a rubber bulb, the electrode being kept in position by vacuum.

III. Esophageal lead. A small electrode, which is swallowed by the patient, is so manipulated that it lies behind the heart. Its distance from the teeth indicates its position in relation to left atrium and ventricle.

IV. His Bundle Electrogram. The electrical events in the AV node, bundle of His, and the Purkinje system, are recorded through an electrode at the tip of a cardiac catheter. The His Bundle Electrogram (HBE) is of special value in various types of heart block.

Procedure. (1) Ask the subject to lie down on the couch comfortably and fully relaxed. Rub small amounts of jelly on the appropriate points and connect the electrodes in position. Switch on the machine and centre the stylus. Adjust the sensitivity calibration control so that the standard 1 mv may produce a deflection of the stylus by 1.0 cm. (2) Record the electrical potentials of the heart with the help of the "Lead Selector" switch in the standard order, namely, Leads I, II, III, lead III at the end of a deep inspiration, aVR, aVL, aVF, and V_1 to V_6 (4 to 6 cardiac cycles for each.) Tear off the paper from the machine and label the leads recorded. Note down the name of the subject and the date.

Discussion. The ECG yields valuable data about the path of the spread of the excitatory wave as it passes from the SA node to its final distribution in the ventricles. Any alterations in its conduction are reflected in the normal pattern of the ECG and thus give a valuable information about the integrity of the heart muscle and its rhythm. The waves produced by the cardiac cycle are named *P*, *Q*, *R*, *S*, *T*, and *U*. The various deflections are shown in Fig. 8.5.

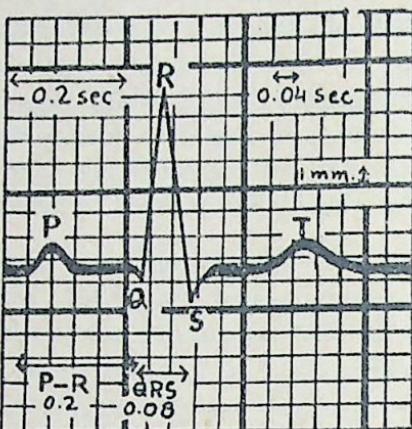


Fig. 8.5 : The waves of the electrocardiogram. To calculate the heart rate per minute from an ECG record, divide 300 by the number of large squares (5 mm apart) between two adjacent R waves.

1. *P wave* : This wave, also called the atrial complex, represents the origin of the impulse in the SA node and its spread (the wave of depolarization) in the atria. Normally, *P wave* is an upward deflection. Maximum normal amplitude=2.5 mm. Maximum normal duration=0.11 second. It is, however, normally inverted in lead aVR. Otherwise, inversion of this wave indicates a new site of impulse origin. *P wave* is replaced by "F" (*Flutter*) waves in atrial flutter and fibrillation.

2. *P—R interval (P—Q interval, if Q wave is present)* : It denotes the time taken by the excitatory wave to pass from the

atrium to the ventricle. i.e., the conduction time of the Bundle of His. The AV node is reached at the top of the P wave. This interval is, however, calculated from the beginning of P wave to the beginning of Q or R. The average duration is 0.16—0.18 sec, the normal maximum duration being 0.2 second. It is prolonged in various types of heart block (atrioventricular dissociation).

3. *Q wave* : This is the first deflection down after the P wave. The max. normal amplitude is 25 per cent of the height of R wave, providing the R wave exceeds 5 mm (some workers use 50 per cent as a criterion). Max. normal duration=0.04 sec. If it is more than 25 per cent of R wave in deep inspiration, in lead III, it is considered pathological.

4. *R wave* : Max. normal amplitude=16 mm in lead I. $R_1 + S_3$ should not exceed 23 mm. The max. normal duration of the QRS complex (ventricular complex) is usually considered to be 0.08 sec, and should not exceed 0.10 sec. Widening of the QRS complex is seen in bundle-branch block.

5. *S wave* : Deep S waves may be seen in normal vertical hearts. The max. normal duration is 0.04 sec.

6. *T wave* : It indicates the repolarization of the ventricular wall. It is upright in leads I and II and variable in lead III. The T wave is not normally inverted in lead I, but 40 per cent of the normals have inverted T in lead III. Max. normal amplitude is 1 mm if R is no less than 5 mm. With tall R wave the T—R ratio should be 1.10. The average duration is 0.27 sec. The QRST is thus 0.4 second.

7. *ST segment* : The RS—T junction is the best place for measuring deviations. Normally, the ST segment is on the isoelectric line. It is abnormal if present above or below the isoelectric line by 2 mm. The ST segment is displaced in myocardial injuries.

8. *U wave* : This is seen just after the T wave in some subjects. It is possibly due to a slow repolarization of the intraventricular contracting system.

Clinical importance of ECG : ECG is of great value in the diagnosis and prognosis of heart diseases like myocardial infarction, (the student may note that the coronary risk factors are : heredity, smoking, hypertension, diabetes mellitus, high blood lipid levels, obesity, high fat diet, emotional stress, and lack of physical exercise), disturbances of cardiac rate and rhythm, heart blocks, atrial and ventricular fibrillations etc.

Einthoven's triangle : The two arms and the left leg form the apices of a triangle (the Einthoven's triangle) which surrounds the heart. The heart is placed, though not symmetrically, in the middle of a volume conductor (body tissues and fluids), and the potentials arising in it are picked up from three points of the triangle. Any changes in the pattern of spread of the cardiac impulse are detected by analysing the contours of various waves in different ECG leads. *Vectorcardiography* deals with such analyses in relation to the electrical axis of the heart in reference to the planes of the body.

Questions : (1) What is ECG ? What is meant by a 'lead' ? Name the various lead systems that are employed in practice. (2) Draw a normal ECG and label its various waves. What is their cause, normal duration and significance ? (2) Comment on the ECG provided. (3) Name some conditions in which ECG is indicated.

Experiment No. : 8.8 **THE VENOUS FLOW**

The flow of blood through the veins of the forearm and the presence of valves in them can be demonstrated by a simple experiment (William Harvey used this experiment in 1628 as

part of the proof for his theory of the circulation of blood). Apply a blood pressure cuff over the upper arm and inflate to 30 to 40 mm Hg ; the superficial veins of the forearm become prominent. Place the tip of the right index finger (*R*) over one of the veins and mark the position of the valve (*V*) above it. With the left index finger, squeeze out blood from this vein towards the elbow, keeping the finger (*R*) in the same position. Note that the segment of the vein between (*R*) and (*V*) remains collapsed and that there is no backward flow of blood from above. The vein above the valve, however, is distended and the valve (*V*) becomes prominent. Keeping the finger (*R*) in position, place the left index finger above the valve (*V*) and try to squeeze the blood downwards towards finger (*R*). Note that the blood cannot be forced backwards across the valve unless a pressure which would be enough to rupture the valve (*V*) is applied.

The valves in the veins play an important role in venous return, especially during muscular exercise (the muscle pump for venous return), and in preventing the exudation of fluid out of the veins in the feet and legs, by breaking the veins into short segments, instead of there being a long continuous column of venous blood. The walls of the veins are quite thin and easily distensible. Though the amount of smooth muscle in the veins is small, considerable venoconstriction occurs due to activity in the adrenergic nerves. Changes in venous tone are important in circulatory adjustments.

Experiment No. : 8.9

THE JUGULAR VENOUS PULSE

The neck veins connect directly with the right atrium, the right external jugular vein being almost in line with it. A careful examination can provide valuable information regarding venous pressure in the right atrium as well as pressure changes

taking place during the cardiac cycle. Jugular venous pulse tracings can be obtained with a polygraph tambour, simultaneously with recordings of ECG, blood pressure, and heart sounds. (Though it is easier to see pulsations in the external jugular vein, using the internal jugular vein is more reliable as an indicator of pressure changes in the right atrium.)

(1) Ask the subject to lie down on a bed with a small pillow under his head and with the chin pointing slightly to the left. Examine the right side of the neck from the clavicle to the angle of the jaw, under good light. Ask the subject to make an expiratory effort against a closed glottis (the Valsalva manoeuvre). This will fill the superficial veins of the neck with blood because of the positive pressure in the thorax impeding venous return. The large vein running close to and parallel with the sternomastoid muscle is the external jugular vein. Mark it with a skin-marking pencil.

(2) Support the upper part of the subject so that he reclines at an angle of about 45° . The external jugular vein shows slight pulsations and two or three small waves may be detected in each cardiac cycle. There is a mean level of blood column in the vein, and the perpendicular height of this level above the right atrium indicates the mean hydrostatic pressure inside the right atrium. In normal healthy subjects, the level is the same as that of the sternal angle, whatever the position of the patient. Sternal angle is thus a convenient point of reference for estimating or measuring the right atrial pressure. Thus, in a healthy person, reclining at 45° , the mean level will be invisible, because it will be below the clavicle. Some slight pulsation, however, may be visible above the clavicle. In cases of right heart failure, where the right atrial pressure is raised, the jugular pulsation is visible and is above the sternal angle, (Fig. 8.6) whatever the position of the patient, i.e., even when the patient is sitting upright.

The jugular venous pulse tracing (Fig. 8.7) shows three positive waves *a*, *c*, and *v*, and two negative waves or descents *x* and *y*. The *a* wave results from atrial contraction. This is followed by the *x* descent, which shows a small *c* wave (this is not usually

visible when the neck veins are inspected). The *c* wave is due to the bulging of the tricuspid valve into the right atrium as the

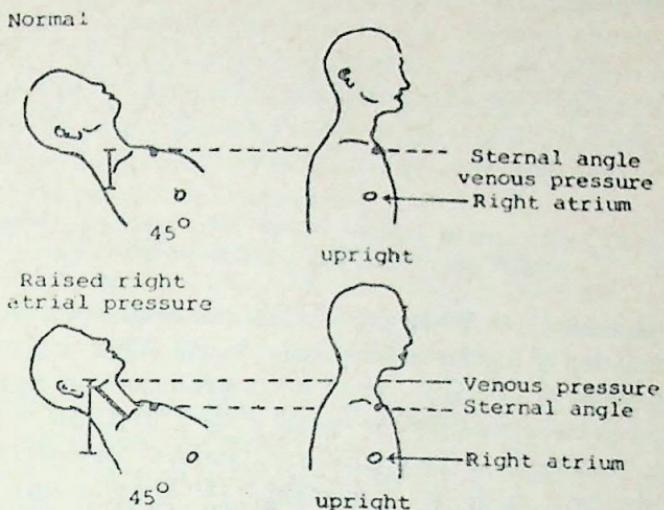


Fig. 8.6 : Jugular venous pressure. In the normal subject, with the back reclining at an angle of 45° to the horizontal, slight pulsations are visible in the jugular vein just above the clavicle. This level is the same as the sternal angle whatever the position of the thorax. The vertical distance between the right atrium and the sternal angle indicates the mean hydrostatic pressure in the right atrium.

In right heart failure (lower figure), the right atrial pressure, and therefore the jugular venous pressure, is raised, the vein is full and shows pulsations even when the patient is upright.

ventricular pressure increases at the start of systole. The *v* wave is due to a passive rise of pressure in the right atrium as it fills with venous blood. As the tricuspid valve opens, blood rapidly enters the right ventricle causing a fall in right atrial pressure—the *y* descent. The *y* descent may sometimes show a small wave.

It may be pointed out that no patient of heart disease has been fully examined until the level of venous pressure in neck veins has been determined. (3) Tilt the subject head down, the jugular veins will be distended with blood in normal subjects.

Note : The carotid arterial pulsation is commonly visible in the neck and should be distinguished from venous pulsation.

Arterial pulsation has a dominant 'outward' thrust and can be easily felt by the examiner by exerting a very slight pressure of the fingers.

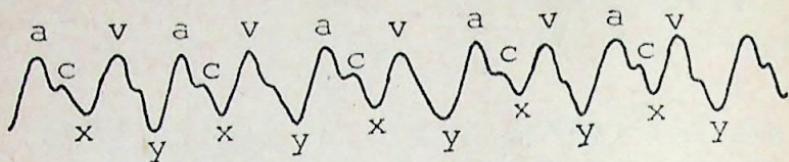


Fig. 8.7 : The normal jugular venous pulse tracing., showing two positive waves—*a*, *c*, and *v*. and two negative waves—*X* and *Y*

Questions : (1) What information can be obtained from the examination of jugular venous pulse ? (2) What is the sternal angle (angle of Louis) ? What is its significance as regards the clinical assessment of jugular venous pulse ? (3) Draw a normal jugular venous pulse tracing, label its various components, and describe their mode of production. (4) What is the normal right atrial pressure ? How does it affect venous return to the heart ?

Experiment No. : 8.10 **VENOUS PRESSURE**

1. Seat the subject on a stool, with the forearm resting on a table, and at the level of the heart. Apply a flat glass cup to the skin of the ventral surface of the forearm over a vein and seal it with collodion. When the seal dries, connect the outlet tube from the cup to a water manometer and a pressure bulb. Increase the pressure in the glass cup till the vein just empties out. Read the manometer at this point. Express the venous pressure in mm of water.

2. Seat the subject on a stool, with the right arm hanging downwards. The veins of the arm will become distended. Gently raise the subject's arm till the veins just above the wrist begin to empty. Determine the vertical height between the wrist and the third costal cartilage at its junction with the sternum (this is the level of the entry of superior vena cava). This distance is an approximate measure of the right atrial pressure. It is raised by Valsalva's manoeuvre and is lowered by Muller's manoeuvre (forced inspiration against a closed glottis).

3. The venous pressure can also be directly measured by inserting a needle (under aseptic conditions) through the skin into a vein in the antecubital fossa, and connecting it to a manometer filled with an anticoagulant solution.

Questions : (1) What type of vessels are veins ? What is their role in cardiovascular dynamics ? (2) What is the pressure gradient from aorta to the right atrium ? (3) What is the effect of gravity on venous pressure ? (4) How does increased venous pressure affect capillary pressure. (5) What is the relation of peripheral venous pressure to right atrial pressure ?

Experiment No. : 8.11
TRIPLE RESPONSE

This is the response of the skin capillaries to mechanical injury. It was first described by Lewis in 1927, and is sometimes called Lewis' response.

Draw a blunt-edged object (a blunt pencil, closed forceps), firmly on the skin of the ventral surface of the forearm. The response will vary with the degree of injury. A moderate pressure will only empty out the skin capillaries leaving a white line in its wake. The reaction differs from person to person, but a

full-fledged response, especially in sensitive skins, consists of the following three stages :

1. **The red line.** It appears in about 8-10 seconds, and is due to the dilatation of the precapillary sphincters produced by histamine and other local metabolic products (polypeptides, substance P., kinins) which are released from damaged tissues (skin), and which produce a passive capillary dilatation and increased blood flow. This response is not mediated by nerves.

2. **The flare.** This is an ill-defined mottled reddish area surrounding the red line, and is due to dilatation of arterioles and precapillary sphincters, resulting in increased blood flow. It is believed to be due to a local reflex, *the axon reflex*, a response in which impulses originating in a sensory nerve are relayed antidromically down the other branches of this nerve, and which supply arterioles. This is perhaps the only example of a physiological effect due to antidromic conduction in the nerve fibres.

3. **The wheal (or weal).** The flare is followed by a local oedema in this area and is due to increased capillary permeability, coupled with increased capillary pressure resulting from dilatation of precapillary resistance vessel. The substances responsible for the wheal are histamine, kinins etc., released from the tissues due to the local damage. Intradermal injection of histamine produces flare and wheal. The exudate contains substantial amounts of proteins which have leaked out of the microcirculation vessels.

Experiment No. : 8.12

CLINICAL EXAMINATION OF THE CARDIOVASCULAR SYSTEM

A patient suffering from heart disease may present with many symptoms, some of which may not appear to be apparently con-

nected with cardiovascular system. Heart disease may be detected during a routine clinical examination, though the patient may not complain of any symptoms. Some symptoms are constantly encountered in heart disease ; a brief resume of which is presented here.

(1) *Dyspnoea* on effort, paroxysmal dyspnoea at rest (attacks commonly called cardiac asthma), or there may be orthopnoea, (2) *Palpitation* : a careful history must be taken. (3) *Cardiac pain* : may present as angina of effort, acute coronary insufficiency, or myocardial infarction. (4) *G.I. symptoms* : there may be loss of appetite or even vomiting. (5) *Respiratory symptoms* : as cough or dyspnoea. (6) *Urinary symptoms* : there may be oliguria in renal failure resulting from heart disease. (7) *Cerebral symptoms* : there may be attacks of syncope i.e., transient loss of consciousness.

The subject of clinical examination is discussed only broadly, indicating the step-wise examination so that the student does not miss any important findings. Standard books on the subject must, necessarily, be consulted.

I. Examination of the Arterial Pulses (see 8.3)

II. Recording of the Blood Pressure (see 8.6)

III. Inspection and Palpation : The subject should be examined in good light, with chest bared, but without unnecessary exposure to chill, both in sitting and supine positions. Notice the shape of the chest and praecordium and observe if there is any dyspnoea or cyanosis. Now look for :

1. The neck veins and pulsations in the neck.
2. Presence or absence of veins on the chest wall.
3. *Cardiac impulse* : Confirm the position of the apex beat and see if there is any deviation.
4. Look for any other pulsations in the suprasternal notch and at the root of the neck. In the neck, carotid artery pulsations are usually strong due to exertion, emotional excitement, or overaction of the heart due to some disease.

5. Pulsations in the thorax may be due to aortic aneurysms. The site will vary, depending upon the location of the aneurysm. In coarctation of aorta, pulsation in the superficial arteries of the thorax may be visible.

6. Pulsations in the epigastrium are common in thin subjects, or are due to nervousness and excitement. These are due to pulsations of the abdominal aorta.

7. *Thrills* : Place your hand over the precordium in the correct manner. If any vibration-like sensation is felt over any region, mark it with pencil. A palpable murmur is called a *thrill* and it has a vibrating quality. As a matter of fact, any sound or murmur which is loud enough is palpable. Notice if the thrill is propagated in any direction. Feel the carotid arteries.

No amount of explanation can make a student understand thrills and murmurs. Only palpation and auscultation in a patient will help him to understand their nature, timing and clinical significance. The loud first heart sound of mitral stenosis is quite often palpable. Semilunar valve closures are often palpable in pulmonary and systemic hypertension.

IV. Percussion : The correct method of percussion is :

1. Place your left middle finger firmly in contact with the skin. Strike its middle phalanx with the tip of the right middle finger 2-3 times. The percussing finger should be relaxed and should not be lifted up more than 3 inches or so and the movement of the hand should be loose and at the wrist and the finger joints and not at the elbow. Furthermore, the percussing finger should lie almost over and parallel to the left middle finger. Practice percussing various objects ; a solid surface, an empty barrel etc. Notice two things while percussing (i) the feeling imparted to the percussed finger and (ii) the sound produced, which differs both quantitatively and qualitatively.

2. Demarcate the area of cardiac dullness, going from right to left, starting over the lungs and moving towards the right border of the heart ; repeat it from left to right, and then, from above the region of the heart. The area of cardiac dullness is

increased in large pericardial effusions. Percussion may help to some extent in cases of aortic aneurysms. In pulmonary emphysema, the area of cardiac dullness may be decreased. X-ray pictures, of course, make the diagnosis easy.

V. Auscultation : Much practice is required to become familiar and proficient with heart sounds and murmurs and their time relationships. It is good practice to palpate the carotid artery while auscultating. The student must learn to focus full attention on one portion of the cardiac cycle at a time. As a routine, the four cardiac areas i.e., mitral, tricuspid, aortic, and pulmonary are auscultated first. It must be understood that the auscultation should not be confined to these areas alone. Look for the following deviations from the normal.

1. The intensity of sounds may differ, both absolutely and relatively to each other.
2. The sounds may be split. This indicates that the *A-V* valves and the semilunar valves are not closing simultaneously.
3. The spacing of heart sounds may be altered.
4. A triple rhythm may be present.
5. Adventitious sounds may be present, and these may occur along with, or replace the heart sounds. Murmurs have a blowing quality, and the time of their occurrence in relation to heart sounds (systolic, diastolic and other murmurs), point of maximum intensity ; their direction of selective propagation, their behaviour during respiration, and their character should be noted, as they are of great help in the diagnosis of valvular diseases. Pericardial friction is also an adventitious sound. It gives an impression of two pieces of dry leather being rubbed together. In children a continuous murmur called "venous hum" can be heard in the neck and upper chest. Listening to prerecorded heart sounds as well as adventitious sounds is of much help.

CARDIAC EFFICIENCY TESTS

Principle : The single and possibly the best test of the efficiency of the heart is the exercise test (exercise tolerance test). Many types of such tests are in use, the objective being to study the effect of exercise on cardiovascular and respiratory systems.

When cardiac efficiency is decreased, exercise causes severe palpitation, respiratory distress or dyspnoea, open mouth, action of alae nasi, and action of accessory muscles of respiration. In patients with ischaemic heart disease, the patient experiences discomfort and pain in the chest and along the left arm. ECG shows typical changes in these circumstances.

Cardiac efficiency tests are indicated in candidates applying for certain jobs, in those buying life insurance, and in some diseases of the heart to assess its functional status and effectiveness of treatment.

Traditionally, the severity of exercise has been categorized into two types : *moderate* and *severe*. In moderate exercise the O₂ consumption rises to about 3 times the resting level while in heavy exercise it rises to 6-8 times the resting value.

In the present experiment the students will study the effect of moderate exercise on blood pressure, heart rate, and respiratory rate and depth. The extent of increase in these parameters and the time after which they return to pre-exercise levels will also be noted. In addition, the effect of posture, deep inspiration and expiration, breath holding and pressure on carotid sinus (expt. 8.5) will be studied.

Apparatus : Sphygmomanometer. Stop watch. Stethoscope.

Procedure : Draw columns as shown in table 8.2 in your note books for entering the observations as and when they are made. The students will work in groups of two, one acting as the subject and the other as the observer.

(1) Record the blood pressure, pulse rate and the rate of respiration in the lying position. These figures will act as control values (or take the values recorded in sitting position as controls):

(2) To study the effect of posture record the above parameters in the supine, sitting and then in the standing position. When studying the effect of posture on blood pressure the time interval between recordings is a critical factor (see below). Enter the figures in your note book.

(3) With the armlet of the sphygomanometer in position on the upper arm and disconnected from the apparatus, hop 20 times on each foot, raising the body 6 to 9 inches off the ground each time. Alternately the subject may do knee bends, or do 'spot running', bringing the thighs to the horizontal position each time, until he becomes mildly breathless.

(4) During the exercise, note if there is any flushing of the face, or respiratory distress. Discontinue the exercise in such an event.

(5) Soon after the exercise is over, record the three parameters in the sitting (or lying) position.

(6) Record the parameters 1, 3, and 5 minutes after the end of the exercise.

(7) Listen to the heart sounds during the waiting periods and note if there is any systolic murmur (these are a normal feature in some individuals when the heart is beating actively).

(8) After the heart rate, blood pressure and respiratory rate have returned to the pre-exercise levels, record these during deep inspiration, and during breath holding some 10-15 seconds before the 'breaking point'.

(9) Study the effect of pressure on carotid sinus as described in experiment 8.5.

(10) Your tutor will record the ECG in one or two students before and after exercise and discuss the tracings with the class.

Observations and Results : (1) *Posture* : In normal subjects there are little changes in the parameters under study in res-

ponse to changes in posture. Immediately after assuming the erect posture (i.e., in the first 8-10 seconds) the blood pressure tends to fall a little but then the baroreceptor mechanism comes into operation and restores it to the normal level or to slightly above normal.

(2) *Exercise* : The heart rate increases by 40 to 50 beats per minute or even more depending upon the severity of the exercise and physical conditioning of the subject. The increase in heart rate and blood pressure is more in those with sedentary habits.

The systolic pressure increases by 15 to 20 mm Hg while the diastolic pressure may remain unaltered or fall a little. The rate and depth of respiration increase.

(3) *Recovery after exercise* : Normally, the parameters under study return to the pre-exercise levels within 1 to 3 minutes, the heart rate recovering more slowly than the blood pressure. The recovery is earlier in subjects who exercise regularly or engage in sports activities.

(4) During deep inspiration the heart rate increases—a normal phenomenon called sinus arrhythmia. During deep expiration it tends to slow down a little. Both blood pressure and heart rate increase during breath holding.

(5) Pressure on carotid sinus causes slowing of heart and fall in blood pressure (see exp. 8.5)

Discussion : The initiation and coordination of movements during exercise is controlled by the nervous system. The extra O₂ and fuel requirements are supplied by a series of complex but appropriate reflex adjustments in cardiovascular and respiratory systems, the autonomic nervous system playing the pivotal role in these.

(a) *Cardiac output* : The cardiac output increases in direct proportion to the increase in O₂ consumption (in moderate exercise it may increase to 12-15 litres per minute while in very heavy exercise it may reach 35 litres or more). The local metabolites in the muscles (eg, K⁺, H⁺, PO₄²⁻, lack of oxygen and

excess of carbon dioxide etc.) cause dilation of the arterioles and precapillary sphincters, thus greatly increasing the muscle blood flow (active hyperemia). The muscle and respiratory 'pumps' increase the venous return to the heart resulting in increased output. In moderate exercise, the increase in cardiac output is mostly due to increase in stroke volume (40-50% increase) and to some extent the heart rate. Increased cardiac sympathetic activity, and later, circulating catecholamines, increase the speed and force of cardiac contraction leading to more complete emptying of the ventricles.

(b) *Heart rate* : Many factors contribute to the increase in heart rate ; These include : psychic stimuli (the rate increases even before the exercise begins), afferent impulses from the mechanoreceptors in the active limbs (muscles, joints ligaments), decrease in vagal tone, increases in cardiac sympathetic activity, reflex activation of pulmonary stretch receptors, and possibly the Bainbridge effect.

(c) *Blood pressure* : In spite of a large increase in cardiac output, the rise in blood pressure is less than might be expected. The reason for this is the fall in total peripheral resistance as a result of vasodilatation in the muscles. Systolic pressure increases by 10-15 mm Hg while diastolic pressure remains unchanged or even falls a little. After exercise the blood pressure may fall temporarily to subnormal levels, presumably due to accumulated metabolites keeping the muscle vessels dilated for a short time. However, it soon returns to normal levels.

(d) *Respiration* : The respiration increases both in rate and depth. The increase in ventilation is in direct proportion to the increase in O_2 consumption. The control mechanisms are so accurate that PCO_2 and PO_2 , remain almost unchanged (that is quite contrary to what the students usually expect), except in severe exercise. Thus lack of O_2 and excess of CO_2 are not the cause of hyperventilation. The following reflex mechanisms summate to increase the ventilation : collaterals from descending motor pathways, limb afferents stimulated by movements, greater sensitivity of peripheral chemoreceptors to CO_2 and O_2 and increased venous return. In prolonged exercise, rise of body temperature also plays a role.

Physical conditioning and benefits of exercise : Both at rest and at any given level of exercise, trained athletes have a large stroke volume and a lower heart rate (due to increased vagal tone) than untrained persons. The size of the athletes' hearts is also larger.

One of the well known benefits of exercise regimens is psychologic, those who exercise regularly "feel better". This is quite evident from the current vogue for jogging and other forms of exercise. There is evidence that regular exercise reduces the incidence and severity of myocardial infarction. Exercise increases the probability that one will remain active and healthy well past middle age. Exercise should primarily be isotonic rather than isometric, because the latter produces a greater increase in cardiac work. Exercise under medical supervision is being used with greater frequency in the treatment of patients with ischaemic heart disease.

Clinical Application of Exercise Tolerance Tests : The objec-

Exercise tolerance tests or "stress testing" as it is usually called, has gained wide acceptance, especially in cases of ischaemic heart disease. In one type of stress testing, cardiac output and intraventricular pressures are measured by cardiac catheterization. In the second form, the ability of the coronary circulation to enhance coronary blood flow in response to increased oxygen demand is tested. ECG is monitored during and immediately after exercise to detect depression of ST segment and changes in T wave. The exercise is done on Master's two step stairs or on a treadmill or on a bicycle ergometer. The tests are standardized with standard external work loads, or by heart rate response or by reaching maximum possible exercise load. In *target heart rate test*, exercise is continued until the subject attains 80 to 90 percent of his predicted maximum heart rate calculated from available tables. Oxygen consumption can be indirectly determined from maximum achievable heart rate (MAHR). A physician must be in attendance throughout the test since the test may have to be discontinued if there is pain in the chest or changes in the morphology of ST segment and T wave.

Special Investigations for Assessment of Cardiac Efficiency : In addition to ECG stress testing, X-Ray of the heart, phonocardiography and serum enzyme studies, many new techniques have been introduced in the recent years. In *radionuclide ventriculography* (RVG), thallium 201 is injected intravenously and since it is concentrated by the normal myocardium, cardiac imaging helps to locate areas of ischaemia. *Echocardiography*, in which ultrasonic sound waves are directed on the praecordium and their reflections recorded, is another non-invasive technique that provides a direct image of the heart. The size of the left ventricle can thus be determined during systole and during diastole and the ejection fraction calculated.

Cardiac catheterization, an invasive technique, is employed when detailed information about the heart and coronary vessels cannot be obtained from the above-mentioned non-invasive techniques. In this procedure, a thin flexible, and radio-opaque catheter is passed into the right or left side of the heart under fluoroscopy. Catheters with special monitoring devices mounted at or near their tips are employed to determine pressures in various chambers, cardiac output, heart sounds, electrical activity, or to collect blood samples for gas analysis, or to inject radio-opaque dyes into the coronary arteries. Coronary arteriography is an essential investigation before coronary by-pass surgery.

TABLE 8.2 Assessment of cardiac efficiency

Name of the subject :

Name of the observer :

	Blood Pressure			Pulse	Respiratory	Pulse/Respi-
	Systolic pressure	Diastolic pressure	Pulse pressure	Rate	Rate	ration Ratio
Control Values (sitting or lying)						
Posture						
lying						
sitting						

standing

Exercise

end of exercise

1 minute after

3 minutes after

5 minutes after

Deep Inspiration

Deep Expiration

Breath Held

Pressure on
Carotid sinus

Questions : (1) What are the indications for exercise tolerance tests ? (2) How would you categorise the severity of exercise ? (3) What is the effect of moderate exercise on blood pressure, heart rate and respiratory rate ? (4) What is active hyperemia ? What is its cause ? (5) What are the factors which increase the venous return during exercise ? (6) What are the factors that contribute to an increase in the heart rate, blood pressure and ventilation ? (7) What is the effect of training on cardiorespiratory responses to exercise ? (8) What is the clinical importance of exercise tolerance tests ?

Nervous System

The short experiments included here are meant to illustrate some of the features, as well as the limitation, of our cutaneous sensations, namely touch/pressure, and pain. Some of these are employed as clinical tests during the neurological examination of a patient.

Instructions. Work in batches of two, one student acting as the *subject* and the other as the *observer*. The observer should ensure that the subject's answers are honest and based on the sensation arising in the end organ under study. While testing a sensation, whether in a student or a patient, it is important not to suggest the response, either verbally or by allowing him to see the test stimulus. Because of the variability in responses to identical stimuli, the test should be repeated enough times to obtain a mean value. In general, for the class work, the test may be repeated 4 to 6 times in order to obtain this value. During any lengthy testing, the attention of the subject should be checked from time to time.

Experiment No. : 9.1

SENSATION OF TOUCH

Draw one inch squares on the palm at the base of the thumb, back of the hand, and the forearm—one hairless area and the other hairy. Divide each square into four squares. If von Frey's hair aesthesiometer is available, it should be used for testing the density of touch spots in the different skin areas. Horse hair or nylon fibres mounted in hypodermic needles also give good result.

Prepare similar grid charts in your notebooks, preferably on a larger scale, and record the positions of touch sensitive and touch-insensitive spots within the squares drawn on the skin. The purpose of the grid is spatial location, and not an indication of spot size.

Compare the sensations when a wisp of cotton is lightly stroked over the palm and over the back of the hand.

1. Touch localisation. The ability to locate the position of a cutaneous sensation means that the subject is able to indicate accurately the site of stimulation on the skin. Test this faculty with the subject's eyes closed. Touch the skin, briefly and lightly with a coloured sketch pen, at different points, like finger-tips, wrist, forearm and in front of leg, and ask the subject to mark the spots touched, with a different colour. Record the differences observed over various regions. Test the ability to recognise the direction of lines drawn on the skin with your finger. Trace out different letters and figures on the extended palm and find out if the subject can identify these.

2. Two-point discrimination. The ability to distinguish two simultaneously applied stimuli as separate, is an important feature of our cutaneous sensory system. Set the Weber's compass (an ordinary pair of dividers can be used) to some separation and then briefly and lightly touch the skin with both points simultaneously. Test the skin areas on the back of forearm, wrist, back of hand, palm and fingertips. Vary the distance between the two points irregularly and retest. Occasionally,

check the response to stimulation with one point. The subject should report 'one', 'two' or 'don't know'. Report the minimum separation discriminated with certainty for each site.

Experiment No. : 9.2
TEMPERATURE

(1) Prepare three jars of water in the order—hot, warm, and cold. The hot water should be as hot as can be easily tolerated by a finger without evoking pain. Dip the right index finger in the cold and the left in hot water. After about half a minute dip both in warm. The warm water will feel hot to the right and cold to the left. (2) Map out 'cold' and 'warm' spots on the skin areas used for touch, using a blunt pithing needle, packed in ice or warmed. The subject should report a sensation of 'coldness' and 'hot' and not when the stimulus can be detected by its pressure. (*Note.* Dry the needle before use and use it only once before recooling it). (3) Take two small test tube containing hot and cold water. Apply these on different areas of skin on the face. The two sensations can be perceived and differentiated easily.

Experiment No. : 9.3
PAIN

This experiment is concerned with the fast component of pain (the first or the pricking pain). It may be noted that very little pressure is required to elicit this type of pain.

The subject closes his eyes, or is blindfolded. Use a mounted needle or a lancet to apply brief, light stimuli within the one inch squares as used before. Map out pain-sensitive areas within these grids. (*Note.* The subject should report when the stimulus elicits pain and not when the stimulus can be detected as touch/pressure.

*Experiment No. : 9.4***VIBRATION SENSIBILITY**

Place the stem of a vibrating tuning fork over the skin of bony and muscular regions. (A low frequency tuning fork should be used). Compare the sensations from the two regions.

*Experiment No. : 9.5***STEREOGNOSIS**

This complex sensation depends on the appreciation of spatial and temporal relationships of the stimuli. It is concerned with the ability of identifying common objects placed in a hand, without the aid of vision. The features of an object, like softness or hardness, dryness or wetness, roughness or smoothness (e.g., the milled edge of a coin) and the dimensions help us in its identification.

Place different but familiar objects (a key, a coin, pencil) one after the other, in the hand of the subject and ask him to name the object and the purpose for which it is used.

*Experiment No. : 9.6***PROPRIOCEPTIVE SENSES**

1. *Sense of position* : Test the sense of position by placing the terminal parts of the limbs (terminal phalanx of a finger or toe) gently, towards or away from the subject, with the eyes closed, and ask him about its position. Place one limb in a particular position and ask the subject to place the other limb in an identical position.

2. *Sense of movement* : Gently move the terminal part of the limb and ask the subject whether that segment is moving or stationary. This sense is closely related to the sense of position.

Testing the position of a flexed or extended small joint is more of a joint sense than position sense which is a grosser sense. The senses of position and movement are tested during clinical examination of the nervous system.

*Experiment No. : 9.7***CLINICAL TESTING OF REFLEXES**

The reflexes which are of importance in clinical neurology can be divided into the following groups :

I. *Superficial reflexes* : (a) Reflexes from skin and (b) Reflexes from mucous membranes.

II. *Deep or tendon reflexes* (tendon jerks).

III. *Visceral reflexes or organic reflexes*.

IV. *Pathologic (or abnormal) reflexes*.

The following points must be remembered in all reflexes :

1. Method of eliciting the reflexes.
2. Response or the result produced.
3. Afferent and efferent paths.
4. Centres (in the central nervous system) for the reflexes.
5. The clinical significance of each reflex.

I. Superficial Reflexes

The stimulation of a particular part of skin or mucous membranes results in the contraction of certain muscles. The reflex arcs for skin reflexes seem to be complex and long, and include a number of internuncial neurones. The afferent impulses appear to be carried up in the posterior white columns and spinothalamic tracts and end somewhere in midbrain, thalamus or even the forebrain. From here, the impulses are carried down by pyramidal and extrapyramidal tracts to the anterior grey column cells. The neural paths for deep reflexes, on the other hand, are simple and short, ending in the spinal cord itself.

A. Skin reflexes. *Plantar reflex (Plantar flexor reflex).* A scratch is given along the outer edge of the sole of the foot, with a pencil or the blunt point of a needle, starting from the heel towards the little toe, and then, along the bases of the toes, medially. In healthy adults, there is a plantar flexion of the toes, especially the big toe, and the ankle is dorsiflexed and inverted. Stronger stimuli may produce withdrawal of the limb. This reflex is mediated by the first sacral segment of the spinal cord.

Babinski's sign (extensor plantar response ; it was named extensor because the movement of the toes is in extension according to anatomical terminology). This was first demonstrated by Babinski in 1896 in cases of upper motor neurone (UMN) lesion. There is dorsiflexion of the big toe followed by extension and/or fanning of the other toes, dorsiflexion of the ankle and flexion of the knee or even hip joint (the stimulus need not be painful). This response has also been called 'Babinski toe sign', 'upgoing

toe', and 'positive Babinski reflex'. With slight lesions, this response may be obtained only from the lateral region while a normal response is obtained from the medial region of the sole of the foot. Babinski sign is a part of the mass flexor reflex (withdrawal reflex) seen in cases of spinal cord transection.

The upward movement of the toes is physiologically a flexion movement since it decreases the length of the limb. The spinal cord favours flexor activity; in UMN lesions, the spinal cord is released from the inhibitory influences from higher centres, hence the dorsiflexion of the toes.

Babinski sign is perhaps one of the most important signs in clinical neurology. It is present in the following conditions:

(i) Upper motor neurone lesions. (ii) Spinal cord tumours. The pyramidal fibres are very sensitive to pressure, so the early appearance of this sign, (iii) In narcosis, coma and immediately after an attack of grandmal epilepsy, (iv) Biochemical disturbances affecting brain e.g., hypoglycemia and (v) In infants, the myelination and proper functioning of the pyramidal tracts is yet incomplete.

The pathway for this reflex is : Afferent nerve=tibial (L—5, S—1) Centre=S—1, 2 ; Efferent nerve=L—4, 5 (extensor hallucis longus, physiologically a flexor muscle, is supplied from these segments).

Equivocal Babinski. When it is not possible to be sure whether the response is plantar flexor or plantar extensor, the stimulus should not be applied repeatedly, one or two scratches should suffice. The reflex may be tested again after a short time, and the big toe watched carefully for any movement.

2. *Epigastric reflex.* Stroking the chest (with a blunt point) downwards from the nipple results in drawing in of the epigastrium on the same side centre=Th—7,8.

3. *Superficial abdominal reflexes.* A light stroking of the skin, preferably directed towards the umbilicus, results in brisk contraction of anterior abdominal muscles lying directly under the stimulus. Centres are—Upper abdominal=Th—8,9,10 ; Middle

abdominal=Th—9,10,11 and lower abdominal=Th—10,11,12. In each case the umbilicus is pulled towards the direction of the stimulus. These reflexes are often difficult to elicit in obese subjects, in anxious patients, elderly persons, and after repeated pregnancies. They are absent in UMN lesions.

4. Cremasteric reflex. Stroking the inner side of upper thigh results in drawing up of the testicle due to contraction of cremasteric muscle. Centre=L—1,2.

5. Gluteal and anal reflexes. They are physiologically akin to abdominal reflexes. A scratch on the buttock produces contraction of the gluteus muscle. Spinal segments concerned are L—4, 5. In anal reflex, there is contraction of external anal sphincter in response to a scratch on the perianal skin. The segments for these reflexes are S—4,5.

B. Mucous membrane reflexes

- 1. Corneal or conjunctival reflex.** Touching the cornea or conjunctiva with a wisp of cotton results in *bilateral* blinking. (the centre of the cornea should never be wiped with the cotton). Afferent path is ophthalmic division of 5th nerve; centre is pons; and the efferent path is 7th nerve (orbicularis oculi muscles). Compare the responses on the two sides. Loss of corneal reflex is often an early sign of a lesion of 5th nerve, and may be present before any cutaneous anaesthesia can be detected.

- 2. Pharyngeal reflex.** (gag reflex). A touch upon the posterior pharyngeal wall with a tongue depressor results in constriction of pharynx. Afferent—9th nerve ; Centre—Medulla ; Efferent—10th nerve.

- 3. Palate reflex.** Touching the mucous membrane covering the soft palate results in elevation of the palate. Afferent=9th ; Centre=Medulla ; Efferent=10th nerve.

II. Deep Reflexes or Tendon Jerks

If the tendon of a slightly stretched muscle is sharply tapped with a percussion hammer, the muscle contracts immediately. Deep reflexes are 'fractionated' stretch reflexes. The stimulus

that initiates the reflex is *the stretch of the muscle spindles and not the tendon receptors*. Impulses originating in the muscle spindles are conducted to the CNS, and pass directly to the motor neurones which supply the same muscle. Elicit the deep reflexes on both sides, and compare their speed, amplitude, and the duration of contraction and relaxation of the muscle in each case.

Commonly, the tendon reflexes are graded as : 0=Absent ; 1=Present (as a normal ankle jerk) ; 2=Brisk (as a normal knee jerk) ; 3=Very brisk ; 4=Clonus OR 0=Absent ; + = Sluggish ; ++ = Present ; +++ = Brisk ; +++++ = Exaggerated.

Ask the patient to relax, and elicit the following reflexes :

1. *Maxillary reflex*. (jaw jerk). Strike the middle of the chin with the tip of middle finger when the mouth is slightly open. The response is a sudden closure of the jaw. It can also be elicited by tapping a pencil laid on the lower teeth. This jerk is sometimes absent in health. Afferent=5th ; Centre=Pons ; Efferent=5th nerve.

2. *Biceps reflex*. Tap the tendon of the biceps, the response is flexion of the elbow. Afferent and efferent paths=musculocutaneous nerve ; Centre=C=5,6.

3. *Triceps reflex*. Tap the tendon of the triceps, with the elbow slightly flexed, the response is extension at the elbow. Afferent and efferent paths=Radial nerve ; Centre=C=6,7.

4. *Radial supinator jerk*. Flexion and supination of the forearm upon striking the styloid process of the radius. Afferent and efferent paths=Radial nerve ; Centre=C=6,7,8.

5. *Wrist reflexes*. Extension or flexion of the wrist occurs when the corresponding tendons are sharply struck with a percussion hammer. For flexion—afferent and efferent path is median nerve ; Centre=C=6,7,8. For extension—afferent and efferent path is Radial nerve ; C=7,8.

6. *Patellar reflex (Knee jerk)*. Tap the patellar tendon, the response is extension at the knee joint. Afferent and efferent path is femoral nerve ; Centre=L=3,4. If the patient can sit,

the leg should be crossed over the other and the patellar tendon identified by palpation.

7. Ankle jerk (Achilles tendon jerk). Dorsiflex the foot slightly with one hand and strike the Achilles tendon with the percussion hammer ; the response is plantar flexion of the foot. Afferent and efferent path is tibial nerve ; Centre=S—1,2.

Sometimes the reflexes are feeble (on both sides) in old and debilitated subjects, and there may not be a perceptible movement at the joint, e.g., knee joint. It is a good practice to place a hand on the quadriceps muscle and feel its contraction while the reflex is being elicited. Reinforcement may have to be used when the reflexes are very feeble, i.e., the subject performs some forceful voluntary action. Ask the subject to clench his teeth, or to close his fists with a force, or to pull his clenched hands, while the reflex is being taken. 'Overflow' of impulses to the gamma efferents is possibly the mechanism of reinforcement.

Clinical uses of reflexes. 1. The reflex is absent if there is any lesion of the reflex arc.

2. In UMN disease the deep reflexes are exaggerated and the superficial lost, and the Babinski sign is present.

3. The level of lesion can be determined because the segments for each reflex are known.

III. Visceral Reflexes.

These include pupillary reflexes, reflexes from the heart and lungs (sinoaortic, Hering-Breuer etc.), deglutition, vomiting, defaecation, micturition and sexual reflexes. The following reflexes are tested clinically :

A. Pupillary reflexes : Before testing these reflexes, note the size, shape and motility of the pupils in every subject.

(a) **Size of pupil** : Compare the size of the pupillary apertures on the two sides, first in dim light and then in bright light. Note whether they are small or large and whether there is any irregularity in their outlines. In normal persons, the size of the pupils is quite variable, being usually large in dark eyes as com-

pared to the light eyes. In old age they tend to become small. Slight difference in size on the two sides may be seen in otherwise normal subjects.

If one pupil is larger than the other, it has to be decided which is the normal one. It is not easy to determine this; however, the pupil which is less mobile in response to light is usually the abnormal one.

(b) *Shape* : See whether the pupil on each side is circular in outline (as is the normal case) or if its outline is irregular. In chronic infections of the iris, the pupil may adhere to the anterior surface of the lens and may become irregular in contour.

(c) *Mobility of the pupil* : Test the reactions of the pupils to light and to accommodation as described below :

(1) *Light reflex* (direct light reflex) : Examine each eye separately with the subject in a shady, indirectly illuminated place. Shine a bright light into the eye being tested (bring the torch from the side of the eye and never from directly in front of the eye as the subject will reflexly close his eyes). There is constriction of pupil almost immediately; then it dilates a little, and assumes a smaller size after undergoing a few oscillations. Switch off the light; the pupil quickly dilates to its previous size. The optic nerve is the afferent path; the centre is in the midbrain (the concerned fibres leave the optic tract before the lateral geniculate body and end in the pretectal region of the midbrain on the Edinger-Westphal nuclei of both sides). The efferent path is the oculomotor nerve.

(2) *Consensual light reflex* (indirect light reflex) : Place a hand between the two eyes and throw light into one eye, observing the effect on the pupil of the unstimulated side. There is constriction of the pupil in the other eye (i.e., both pupils constrict). The constriction of the pupil on the unstimulated side is called the *consensual or indirect light reflex*. This response is due to crossing of some of the fibres in the optic chiasma and their termination on the oculomotor nuclei of both sides. Switch off the light and note that both pupils dilate. The consensually-mediated reaction is more active than the direct reaction of a

pupil in some lesions of the optic nerve. Afferent=2nd nerve ; centra=midbrain ; efferent=oculomotor nerve.

(3) *Accommodation reflex* : When one looks at a near object, the pupils constrict, the eyes converge, and the lenses becomes more convex. These three responses constitute *accommodation reaction*.

Hold up your index finger close to the subject's nose and ask him to look at a distant object (or the far side of the room). Then ask him to quickly focus his eyes at your finger. As the eyes converge the pupils constrict (if the patient is unable to see ask him to "look" at his finger held in front of his eye). Afferent—2nd nerve ; centre=visual cortex ; efferent=oculomotor nerve.

In Argyll-Robertson pupil, the accommodation reflex is present but the light reflex (both direct and consensual) is lost, the lesion (neurosyphilis) being located in the pretectal region of the midbrain. The abnormality is bilateral though it is usually more pronounced on one side.

The term *hippus* is applied to a rhythmic dilatation and constriction of the pupil, either occurring spontaneously or in response to light. It has no clinical value, though it may be quite prominent in retrobulbar neuritis.

In *Horner's syndrome* (paralysis of cervical sympathetics which are pupillodilator fibres) the pupil is constricted and there is no dilatation when the eye is 'shaded.' There is slight drooping of the upper eyelid (ptosis). Ciliospinal reflex (see below) is also absent.

(4) *Ciliospinal reflex* : Pinch the skin of the neck on the back ; the response is dilatation of the pupil. Afferent=sensory nerve ; centre=thoracic 1-2 segments ; efferent=cervical sympathetic.

B. Oculocardiac reflex. Press over the eyeball gently with a thumb, the response is slowing of the heart. Afferent=5th ; Centre—Medulla ; Efferent=10th.

C. Carotid sinus reflex. Press over the carotid sinus in the neck (on one side only, never on both sides simultaneously), the response is a slowing of the heart and fall in blood pressure. Afferent=9th; Centre=Medulla; Efferent=10th. This reflex is hyperactive in certain persons with marked vasomotor instability, and in whom, slight stimulation of this sort may produce fainting (carotid sinus syncope).

D. Bulbocavernosis reflex. Pinching the dorsum of glans penis results in contraction of bulbocavernosis. Centre=S—3,4.

E. Sphincter reflexes. These include the reflexes essential for swallowing, micturition, and defaecation. Increased pressure inside the concerned viscera results in complex muscular movements. It must be confirmed from the patient if there is any difficulty in swallowing, (dysphagia) or regurgitation of food through the nose. Any interference in the normal micturition and defaecation reflexes may result in incontinence.

The various reflexes are summarized in table 9.1.

IV Pathological Reflexes

This group includes some primitive responses which occur only with lesions of the UMN. Normally they are suppressed by cerebral inhibition. The reflexogenic area is widened in upper motor neuron lesions, and the Babinski-like response is obtained from widespread areas.

(1) Babinski's sign. (2) Gordon's leg sign : Babinski-like response upon squeezing calf muscles. (3) Oppenheim's sign : Babinski-like response produced by a firm downwards stroking of the anterior aspect of tibia. (4) Chaddock's sign : similar response as above obtained by stroking the malleolus. (5) Hoffmann's sign (finger jerk) : Flick the distal phalanx of the index finger of the patient with your thumb and finger, the response is a clawing movement of the fingers along with the thumb.

There are other such pathological reflexes which can be tested
Note. If the plantar reflex is extensor, the other tests are superfluous ; if it is equivocal or flexor in type, the other tests cannot be taken as substitutes. If the examiner is in doubt

TABLE 9.1 : Summary of reflexes tested clinically

Reflexes (1)	How Elicited (2)	Response (3)	Afferent Path (4)	Centre (5)	Efferent Path (6)
SUPERFICIAL REFLEXES					
(A) Skin Reflexes					
1.	Plantar	Scratch on medial aspect of sole	Plantar flexion of toes	Tibial S-1,2.	Tibial
2.	Epigastric	Scratch on chest down from nipple	Drawing in of epigastrium on same side	Th-7,8	Th-7,8
3.	Upper abdominal	Scratch on upper abdomen	Contraction of the abdominal muscles	Th-8,9,10	Th-8,9,10
4.	Middle abdominal	Scratch on abdomen near umbilicus	Contraction of the abdominal muscles	Th-9,10,11	Th-9,10,11
5.	Lower abdominal	Scratch on lower abdomen	Contraction of the abdominal muscles	Th-10,11,12	Th-10-11,12 Th-10,11,12

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)
6. Cremasteric	Scratch on upper medial thigh	Drawing upwards of the testicle	Femoral	L-1	Femoral
7. Gluteal	Scratch on buttock	Contraction of gluteus muscle	L-4,5	L-4,5	L-4,5
8. Anal	Scratch on skin near anus	Contraction of anal sphincter	Pudendal	S-4,5	Pudendal
(B) Mucous Membrane Reflexes					
1.	Corneal or Touch on cornea or conjunctival conjunctiva with cotton	Closure of eye	Cranial V	Pons	Cranial VII
2.	Pharyngeal	Touch on pharynx	Constriction of pharynx	Cranial IX	Medulla
3.	Palate	Touch on soft palate	Elevation of palate	Cranial IX	Medulla
DEEP REFLEXES					
1.	Maxillary (jaw)	Tapping on middle of jaw	Closure of mouth	Cranial V	Pons
2.	Biceps	Tapping on biceps tendon	Flexion at elbow	Musculo-cutaneous	C-5,6
(Contd.)					

(1)	(2)	(3)	(4)	(5)	(6)
3. Triceps	Tapping on triceps tendon	Extension at elbow	Radial	C-6,7	Radial
4. Radial supinator	Tapping on styloid process of radius	Flexion and supination of forearm	Radial	C-6,7,8	Radial
5. Wrist (flexion)	Tapping on flexor tendons of wrist	Flexion of wrist	Median	C-6,7,8	Median
Wrist (extension)	Tapping on extensor tendons of wrist	Extension of wrist	Radial	C-7,8	Radial
6. Patellar	Tapping on patellar tendon	Extension at knee	Femoral	L-3,4	Femoral
7. Ankle (Achilles)	Tapping on Achilles tendon	Plantar flexion of foot	Tibial	S-1,2	Tibial

VISCERAL REFLEXES

(A) Pupillary Reflexes

1. Light (direct) Shining of light on retina in one eye
Constriction of pupil on that side
2. Light (indirect or con-sensual) Shining of light on retina in one eye
Constriction of pupil on other side

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)
3. Accommodation	Subject looks on finger held in front of one eye	Constriction of pupil in that eye	Cranial II	Occipital cortex	Cranial III
4. Cilio spinal	Pinching of skin on back of neck	Dilatation of pupil	Sensory nerve	Th-1,2	Cervical sympathetic
(B) Oculocardiac	Pressure over eyeball with thumb	Slowing of heart and fall in blood pressure	Cranial V	Medulla	Cranial X
(C) Carotid sinus reflex	Pressure over carotid sinus on one side	Slowing of heart and fall in blood pressure	Cranial IX	Medulla	Cranial X
(D) Bulbocavernosus	Pinching dorsum of glans penis	Contraction of bulbocavernosus	Pudendal	S-2,3,4	Pelvic autonomic
(E) Sphincter reflexes	Distension of bladder or rectum	Emptying of bladder or rectum	Pudendal	S-2,3,4	Pudendal and autonomies

regarding the nature of the response, an involuntary flexion of the leg at ankle knee and hip, after a series of pinpricks, is a valuable confirmation of an extensor plantar reflex.

TABLE 9.2 : Differences between Upper and Lower Motor Neuron Lesions

	<i>Upper Motor Neuron Pyramidal and extra- pyramidal</i>	<i>Lower Motor Neuron Anterior horn cell type including peripheral nerve injuries</i>
1. Loss of power	Incomplete	Complete
2. Extent of paralysis	More extensive and equal involvement of muscles of a limb.	Less extensive and unequal involvement.
3. Atrophy and wasting	None or slight ; generalized ; due to disuse.	Marked, focal. May involve 70-80 per cent of muscle mass.
4. Muscle tone	Increased (rigidity).	Decreased (flaccidity)
5. Reflexes (a) Deep (b) Superficial, abdominal	Exaggerated.	Absent or diminished
	Absent or diminished on the side of involvement.	Unaffected unless thoracic ant. horn cells are affected
6. Pathological reflexes		
Babinski	Present	Absent
Gordon's	Present	Absent
Oppenheim's	Present	Absent
Hoffman's sign	Present	Absent
7. Muscle fasciculation and fibrillation	Absent	Often present
8. Electrical changes in muscle	Normal reactions to Galvanic and Faradic current	Partial or complete reaction of degeneration in the involved muscles
9. Vasomotor phenomena	Mild	Marked
10. Associated movement, e.g., Strumell's sign	Present	Absent

Ankle Clonus. Hold the leg up by placing a hand under the popliteal space, and dorsiflex the foot by a quick and forcible movement. A continued rapid flexion and extension of the foot occurs in UMN lesions.

Patellar Clonus. (Trepidation sign). Keep the leg in extension and relaxed. Depress the patella forcibly with a quick movement, by holding it from the sides with thumb and fingers. A rapid up and down movement of the patella is seen in upper motor neuron lesions. The ankle and patellar clonus are characteristic of conditions in which there is increased gamma motor neuron discharge. (as in UMN lesions).

The differences between upper motor neuron lesions and lower motor neuron lesions are listed in Table 9.2

Questions : (1) What is reflex action ? Describe the components of a reflex arc. (2) How are reflexes classified ? Give examples of each type. (3) What are the main points that must be considered while eliciting any reflex ? (4) While eliciting a deep reflex, we tap the tendon of muscle and the muscle contracts ; are the receptors for the concerned reflex located in the tendon or the muscle, i.e., are the receptors Golgi tendon organs (located in the tendon) or the muscle spindles (located in the muscle) ? (5) What is Babinski sign ? What are the conditions in which it is present ? What does it signify ?

Experiment No. : 9.8

CLINICAL EXAMINATION OF THE CRANIAL NERVES

A sound knowledge of the anatomy and physiology of the cranial nerves is essential in order to understand the logic of methods employed for testing these, and the clinical significance of any abnormalities that may be detected.

I. First or Olfactory Nerve

Take small amounts of oil of peppermint and oil of cloves in two small test tubes. Bring these near each nostril separately, one after the other, and ask the patient if he can identify the smell. Irritants such as ammonia and acetic acid should not be used as they also act through the trigeminal nerve. It should be confirmed before the test that the patient is not suffering from common cold. Loss of sense of smell is called anosmia. Ask the patient if he has any hallucinations of smell. The olfactory area of the cerebral cortex lies in the uncus of the parahippocampal gyrus.

II Second or Optic Nerve

Any errors of refraction should first be corrected. There should be no opacity of the optic media and each eye should be tested separately. Investigate three functions—(A) Acuity of vision, (B) Field of vision, and (C) Colour vision.

(A) Acuity of vision. The acuteness of vision may be much decreased. Confirm first whether light can be perceived. If the impairment is not very gross, do the 'finger—counting' test. This should be done at varying distances. For more accurate assessment of the acuity of vision for distant and near objects, use the Snellen's test charts and Jaeger's test types. Refer to experiment 10.6 for the details of these tests.

(B) Field of vision. The part of the outside world which is visible to one eye, when it is kept fixed on one object or point, is called field of vision for that eye. It is restricted medially by the nose, above by the orbital margin, below by the cheek bones, but it extends more than 90° on the lateral side. An approximate idea of the peripheral field of vision can be obtained by the 'confrontation test', but an accurate assessment and a permanent record can be obtained only by a perimeter (Refer to experiment 10.7 for perimetry).

Confrontation test. Ask the subject to sit opposite you at a distance of about 3 feet. When testing his left eye, he places his cupped right hand over his right eye, and with the left eye he

fixes his gaze on your right eye. Instruct him *not to move his eye in any direction*. Cover your left eye with your left hand. Extend your right arm full length and hold your hand midway between yourself and the subject. Keeping the fingers moving, bring your hand nearer until you can yourself perceive the finger movements 'with the tail of your eye.' At this time, ask the subject if he can catch the movement. If he fails to see the fingers, continue bringing the hand nearer, until he can. Test the peripheral field of vision in this manner—upwards, downwards and from the left side. Test his field for a stationary object in a similar manner by asking the subject to indicate when he sees your finger held at rest. Only gross changes in the field of vision can be detected by this method. Scotomas (blind areas within the field of vision) are impossible to locate.

(C) Colour vision. (i) Holmgren's wools : Place small lengths of woolen threads of different colours and hues in a heap on the surface of a table (white or neutral background), in good light. Pick up a test skein and ask the subject to pick up those wool threads from the heap which are like the test skein, whether they are of a darker or lighter shade (or hue). *He is to match the colour only and not to name it.* Test him first with a pure pale green thread and then with a purple. Note if there is red-green blindness (or weakness); blue-yellow blindness (or weakness), or total colour blindness. (ii) Ishihara's pseudoisochromatic plates are available in the form of a book, and the instructions should be read carefully before testing the colour vision. The plates are so constructed that a subject with normal colour vision will read one number, made up of dots of different colours and size against a background of differently coloured dots, while a person with defective colour vision will read a different number on the same plate. For example—number 8 will be read as 8 by a normal person and as 3 by a subject with defective colour vision. (see colour vision in experiment 11.8).

III, IV, VI, Third Fourth and Sixth Nerves

The oculomotor (3rd nerve), trochlear (4th nerve) and the abducent (6th nerve) are tested together as they innervate the external ocular muscles which move the eyes. The 6th nerve

supplies the lateral rectus, the 4th nerve innervates the superior oblique, and the 3rd nerve supplies all the other extraocular muscles, along with the sphincter pupillae, ciliary muscle (the muscle of accommodation) and the levator palpebrae superiors.

1. Ask the patient to look at your finger held at a distance of about 2 feet from his eyes. Notice if there is any squint (*strabismus*). Ask the patient if he has double vision (*diplopia*) or gets attacks of vertigo.

2. Test for pupillary light reflexes and the convergence accommodation reflex. Notice the size, shape, and mobility of the pupil.

3. Fix the chin of the patient with left hand and ask him to follow the movements of your right forefinger with his eyes. Move your finger in the cardinal directions. The eyes move normally 50° outwards, 50° inwards, 33° upwards, and 50° downwards. Test the rotatory movements of the eyes also. Observe if there is any limitation of movement in any direction.

Usually the signs of involvement of one or more of these nerves are : (i) Pupillary abnormalities, (ii) Presence of diplopia, (iii) Defective movements of the eyeballs, and (iv) Presence of a squint.

Skew Deviation of the eyes i.e., one eye is directed upwards and the other downwards—is seen in diseases of cerebellum, labyrinth, and 8th nerve.

V. Fifth or Trigeminal Nerve (Mixed)

Both the motor and the sensory functions are tested.

Motor Functions. 1. Ask the patient to clench his teeth—The masseter and temporal muscles contract, and should become equally prominent on either side. Confirm by placing your hands on the muscles. The muscles will fail to become prominent if there is paralysis on that side. 2. Ask him to open his mouth—the jaw will deviate to the side of paralysis, the healthy lateral pterygoid muscles pushing it to that side.

Sensory Functions. Test the sensations of touch, pain and temperature over the entire face and over the anterior two-thirds

of the tongue (the taste sensation from this part of the tongue is carried by the facial nerve).

Test the corneal reflex on both sides because the trigeminal nerve forms the afferent path of this reflex. As already mentioned, loss of corneal reflex is one of the early signs of 5th nerve lesion.

VII. Seventh or Facial Nerve (Almost Purely Motor)

1. Ask the subject to wrinkle the skin on his forehead.
2. Ask him to shut his eyes as tightly as possible ; try to open his eyes, first one and then the other. If the orbicularis oculi is normal it is impossible to open the eye against the subject's wish. In lesions of 7th nerve, the patient cannot close the affected eye at all, or if the eye is closed, the eyelashes are not so deeply buried in the face as on the normal side. Normally, as the eyes are tightly shut, the corners of the mouth are drawn upwards. But in paralysis of the lower part of the face, the corner of the face is not drawn up.

When one attempts to shut the eyes tightly, the eyeballs roll upwards—this is a normal response and is called *Bell's phenomenon*. In B:ll's palsy (see below) as the patient is asked to close his eyes, the upward movement of the eyeball becomes obvious because closure of the affected eye is not possible.

3. Ask the subject to smile or show his upper teeth, or to whistle. When there is paralysis of the facial muscles on one side, the angle of the mouth is drawn towards the healthy side.

4. Ask him to inflate his mouth with air and blow out his cheeks ; then tap each inflated cheek with a finger ; air escapes more easily on the affected side.

5. Taste sensation : Though the seventh nerve is almost entirely a motor nerve, the taste fibres from the anterior two-thirds of the tongue pass from the lingual nerve into the chorda tympani and then through the geniculate ganglion of the facial nerve and the nervus intermedius of Wrisburg into the medulla to enter the tractus solitarius. It has been suggested that these

taste fibres sometime run in the maxillary division of the trigeminal nerve ; this however, is rare. The sensation of taste should always be tested whenever a lesion of a cranial nerve is suspected.

How to test the taste sensation : Use strong solutions of sugar and common salt and weak solutions of citric acid and quinine in order to test the sensations of 'sweet', 'salt', 'sour' and 'bitter' respectively. Ask the subject to protrude his tongue and dry the surface with clean cloth. Apply a small amount of sugar solution, on one side of the midline, with a clean toothpick, and ask him : Is this 'salt' ? If the taste sensation is normal he will shake his head. Try salt and then the others in the same manner, rinsing the mouth each time. The tongue should be kept out during the test and not withdrawn. This is to avoid the spreading of the test substance into the posterior part of the tongue since taste from this region is carried by the 9th cranial nerve. Test the taste sensation on the posterior part in similar manner. If the subject can write, then he should write down the taste accordingly. The loss of sense of taste is called *ageusia*. The patient should always be asked whether he experiences any abnormal test sensations. These, like hallucinations of smell, may form the aura of an epileptic fit, especially in cases of temporal lobe epilepsy.

Supranuclear and infranuclear lesions of facial nerve : The chief difference between supranuclear and infranuclear lesions of the facial nerve is that in the former, the lower part of the face is chiefly affected, while in the infranuclear lesion (Bell's palsy), both the upper and the lower parts of the face are equally affected (paralysed).

VIII. Eighth or Vestibulocochlear Nerve (Sensory)

This nerve has two components ; the *cochlear division*, which is concerned with hearing, and the *vestibular division*, which supplies the vestibule and the semicircular canals, and is concerned with posture and equilibrium.

Tests of hearing. Test each ear separately. Confirm that the external auditory canal is waxfree. Use the whisper test and the tuning fork tests as described in Chapter 11.

Tests of vestibular functions. The semicircular canals can be stimulated easily.

Barany's caloric test Syringe the external auditory meatus with water at 30°C or 44°C, while the head is tilted back 60° and pointing to the opposite side, and upto the ceiling (the horizontal canal is placed vertical). The endolymph in the canal moves down by convection currents produced by cold-water syringing. Note the following :

1. Nystagmus.
2. Past pointing. If the subject is asked to touch a given point on a tape held in front of him (when the eyes are closed), the arm deviates out towards the stimulated side.
3. If asked to stand, there is a tendency to fall towards the stimulated side. The subject complains of giddiness and nausea, and may even vomit. All these reactions indicate normal functioning of vestibular apparatus. A patient suffering from vestibular disease complains of paroxysmal attacks of vertigo, tinnitus, nausea and vomiting.

IX. Ninth or Glossopharyngeal Nerve (Mixed)

This nerve is motor for the middle constrictor of the pharynx and the stylopharyngeus muscle, and sensory for the posterior third of the tongue (taste fibres also) and the mucous membrane of the pharynx. Paralysis of 9th nerve alone is rare.

1. Test the pharyngeal reflex.
2. Test the taste sensation of the posterior third of the tongue.

X. Tenth or Vagus Nerve (Mixed)

It is motor for the soft palate (except tensor palati), pharynx and larynx. It is also motor and sensory for the respiratory passages, the heart and most of the abdominal organs (motor parasympathetic). Paralysis of this nerve is evident clinically only through its palatine and laryngeal branches.

1. Ask for the history of regurgitation of fluids through the nose during swallowing. This is due to total paralysis of the soft palate. Use a tongue depressor to watch the movements of palate when the patient is asked to say 'Ah'. If one side is paralysed, the soft palate on that side will remain flat and immobile. In bilateral paralysis, the entire soft palate remains motionless. Conclusions drawn from the position of uvula are usually unreliable, since it may normally be deviated to one side.

2. Laryngoscopy is done to note the position of true vocal cords.

XI. Eleventh or Accessory Nerve (Motor)

This nerve is motor to sternomastoid and upper part of trapezius muscle,

1. Press on the shoulders from behind and ask the patient to shrug his shoulders. Note any weakness or paralysis of the upper part of the trapezius.

2. Ask the patient to turn his head first to one side and then to the other, against resistance, by placing your hand on either side of the chin alternately.

XII. Twelfth or Hypoglossal Nerve (Motor)

It is purely motor to the muscles of tongue, and depressors of the hyoid bone.

1. Ask the patient to push out his tongue as far as possible. If the 12th nerve is paralysed, the tongue is pushed over to the side of the lesion by the contraction of the muscles on the healthy side. Ask him to move the tongue from side to side, over the lips and against the walls of the cheeks. A finger may be placed on a cheek while the patient presses against it with tongue through the wall of the cheek.

2. Note if there is any fasciculation, wasting, or tremor. Wasting and tremor indicate a nuclear or infranuclear lesion (lower motor neurone).

Experiment No. : 9.9

CLINICAL EXAMINATION OF THE NERVOUS SYSTEM

The main purpose of the examination of the nervous system is to determine the nature of disease and its location. Detailed history taking is of prime importance. The examination should be conducted along the following lines

I. Intellectual and Mental Functions.

Note the following :

1. Appearance and behaviour. Well-groomed or unkempt : hair, nails, hands ; disturbed or agitated or terrified ; whether the attention wanders ; any flight of ideas, thought blockage ; whether he uses strange words, or whether normal words are mixed together in an odd fashion.
2. Emotional state. Note if the mood is depressed or elevated, or if there is flattening of emotions. Does he live in and enjoy a world of his own ? Does he appear confused or perplexed ? In depersonalisation, things and people appear changed, and the patient often complains about it.
3. Delusions and hallucinations. Delusions are false beliefs while hallucinations are false impressions.
4. Orientation in place and time. Ask the patient about the date and month, the year, and whether he is in a hospital or in his home.
5. Clouding of consciousness. Ask him about events occurring around him.
6. Memory. Test for recent and past memory by asking questions. In brain injuries, for example, recent memory is affected much more than past memory.
7. General intelligence. This will be evident during the history taking. Ask for educational history and work record.

One of the simple tests is to ask him to continue deducting 7 from 100. Tests of reasoning and 'absurdities test' can give a fair idea of the intelligence.

II. Speech

Look for defects of articulation. Test for various types of aphasia. Give him various objects and ask him to name them, and the purpose for which they are used.

III. Cranial Nerves

See the testing of cranial nerves.

IV. Motor Function

Examine the following aspects of motor system :

1. **Bulk of muscles.** The state of nutrition is examined by inspection and palpation of various muscles, and comparing these on the two sides. In muscular atrophy, the muscle mass decreases and the muscle gives a soft and flabby feeling. Atrophy may be general, following a prolonged illness. Physical exercise increases the bulk (hypertrophy) of the muscles. In some diseases of the muscles (dystrophies), the muscle mass increases (pseudohypertrophy), but these bulky muscles are weak despite their size. Calf and buttock muscles are especially involved.

2. **Muscle tone.** This is the state of slight tension in which the healthy muscles are continuously maintained. Test for increased tone (hypertonia), or decreased muscle tone (hypotonia), at different joints by passively moving the parts of the limb and assessing the resistance offered by the muscles.

3. **Strength of muscles.** Assess the extent of weakness or paralysis by observing the patient while he walks, sits, or stands up from supine position. If confined to bed, assess the force of the hand grip, and ask him to move parts of the limbs actively against resistance offered by you. Test the muscles of thumb, fingers, wrist, forearm, shoulder, trunk, and the lower limbs. Compare the muscular power on the two sides.

4. **Coordination of muscular activity.** Ask the patient to extend his arm and to touch the tip of his nose with the index

finger (finger-nose test). Tell the patient to pronate and supinate his forearms rapidly, with the elbows flexed and kept by the side of the body. The coordination in the lower limbs is tested by asking the patient to walk in a straight line ; look for deviation to one or the other side. If he is unable to walk, ask him to place the heel of one foot over the knee of the other leg and to move the heel along the anterior surface of tibia towards the ankle (heel-knee tests). Other tests for muscular coordination include threading a needle or describing circles in the air with the big toe and index finger.

Romberg's sign : It is a special test for the coordination of lower limbs. The subject is asked to stand with feet close together and then close his eyes. If he begin to sway and tends to fall the test is called Romberg's sign. It means that due to lack of sensory information from the lower limbs he is unable to maintain balance without the aid of vision. A lesion of dorsal spinal roots or posterior columns (as in tabes dorsalis) will produce Rombergism.

5. Any abnormal movements. Tremor (fine or coarse), chorea, athetosis, ticks etc., if present, should be recorded on the history sheet.

6. Gait of the patient. Ataxic, hemiplegic, waddling, spastic, or a festinant gait may be present. Note if there is any foot drop.

V. Sensory Functions

Test the following sensations :

1. **Touch.** Test with a wisp of cotton, on identical points on the two sides of the body. Test for two-point discrimination in addition to touch appreciation and localisation.

2. Use a pin or a needle for pricks on the skin. Test both sides for pressure pain from muscles by pressing upon these with a thumb, or by squeezing them.

It is a common practice to test touch and pain, over identical locations, with cotton in one hand and a needle in the other, and asking the patient to indicate 'cotton' or 'needle'.

3. **Sense of position.** Test the sense of positon, in both limbs, as described earlier.
4. **Sense of movement.** Test this sense without the aid of vision by the subject.
5. **Stereognosis.** Test this ability with different objects as explained in experiment 9.5. Loss of this faculty is termed astereognosis.
6. **Vibration.** Use a tuning fork of low frequency for testing the sense of vibration.
7. Ask the patient if he experiences any numbness, or heat, or chill, or a feeling of 'insects crawling over the body', or a feeling of 'pins and needles'. This condition is called parasthesias.

VI. Reflexes

Test the various superficial, deep and visceral reflexes described previously.

Questions : (1) How would you proceed to examine the motor functions of the upper/lower limbs ? Which parts of the brain are concerned with motor functions ? (2) How would you proceed to examine the sensory functions in the upper/lower limbs of the subject provided ? (3) Name the cutaneous receptors ? What is a receptor potential ? Which sensation shows most rapid adaptation (4) Name the two major ascending systems of the central nervous system. Which sensations are carried by these tracts ? Compare the features of the two sensory paths. (5) What is the role of thalamus in sensation ? Which sensations are perceived (experienced) here ? What is thalamic syndrome ? (7) Name the Brodmann's areas concerned with motor and sensory functions. What is aphasia ? (8) Where is pain perceived ? What is the role of cerebral cortex in pain ? What are the stimuli of pain ?

Vision

Experiment No. : 10.1

**MECHANICAL STIMULATION
OF THE EYE**

Close your eyes and press the outer corner of an eye with the index finger. The pressure produces an impression of a dark circular spot, surrounded by a bright circle, in the field of vision directly opposite the point of pressure. These visual sensations are called 'pressure phosphenes' and are produced by inadequate retinal stimulation.

This experiment supports the Mulleer's 'Law of Specific Nerve Energy', which states that different varieties of stimuli applied to a sensory organ always produce the sensation peculiar to that receptor. In the case of retina, the natural stimulus, light, requires minimum of energy to stimulate the rods and cones.

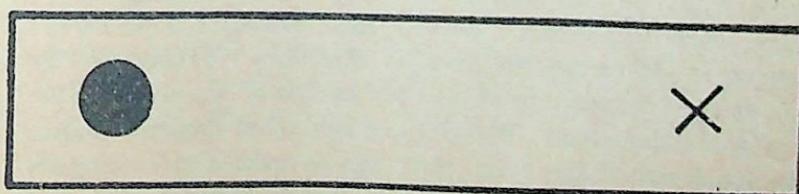


Fig. 10.1 : Mariotte's experiment to demonstrate the physiological blind spot of the eye.

Experiment No. : 10.2

PHYSIOLOGICAL BLIND SPOT

The 'optic disc' is the region where the optic nerve fibres leave the eye and the retinal blood vessels enter. It is located at the point 3 mm medial to and slightly above the posterior pole of the eye (the location of macula lutea). As there are no receptors in this region, any image formed on this is not 'visible'. The optic disc is, therefore, called the blind spot. This region is the only place in the body where blood vessels can be directly visualised (with an ophthalmoscope). Consequently, ophthalmoscopy is very valuable in clinical practice.

1. Close your left eye and hold the figure (Fig. 10.1) in front of your right eye. Fix your right eye on the circle on the left of the figure. Bring the book slowly towards your eye until, at a certain distance (approximately 25 cm) the cross disappears. At this moment the image of the cross is falling on the blind spot (the region of the exit of optic nerve fibres and the entry of retinal vessels) where there are no rods or cones. Bring the book nearer ; the circle reappears. Draw the figure on your note book.

2. *Mapping the blind spot* Mark a cross in the centre of a white paper sheet. Prepare a 12 inch long and one inch wide strip of paper and mark a 3mm black spot at one end. Rest the subject's chin on a couple of books placed on a table. Hold the paper sheet in front of his right eye and ask him to fix his gaze on the cross. Holding the paper strip from the unmarked end, move it over the paper sheet so that the dot travels from lateral to medial side towards the cross. Make a mark on the sheet, with a pin, through the dot when the dot disappears and again when it reappears. Repeat this procedure with the dot moving in the vertical and oblique directions. Finally, join the pin marks with a pen to obtain an outline of the projected image of the blind spot. By using the method of similar triangles, the actual size of the blind spot can be calculated—taking the distance between the retina and the nodal point of the eye at 15 mm.

*Experiment No : 10.3***NEAR POINT**

Hold a pencil in front of one eye (the other being closed), and bring it slowly nearer till it can no longer be seen clearly. Measure the distance between the pencil and the eye. If glasses are worn, determine the near point without glasses.

Accommodation i.e., the process by which the curvature of the lens is increased when we focus the eyes on near objects, is an active process and requires the contraction of the ciliary muscle. This muscle is one of the most frequently used muscles in the body and can, therefore, tire, as is evident from daily experience.

With increasing age, the lens becomes less elastic, and the near point recedes. Presbyopia is the common error of refraction encountered in middle age, when the person has to hold the reading material farther and farther away from the eye as the age advances. The error is corrected with convex lenses.

*Experiment No : 10.4***THE NEAR RESPONSE**

Seat the subject near a window, in good light, and ask him to fix his gaze at a distant object. Bring your finger in front of his eyes and ask him to focus the eyes on it. There is convergence of eyes, constriction of pupil, and increase in the curvature of the lens. This three-part response is called the near response.

*Experiment No. : 10.5***SANSON IMAGES**

If a phakoscope is available it should be used for the demonstration of the images, but a simple experiment can, however, be conducted in a dark room.

Hold a candle on one side of the subject's eye and observe the images from the other side. The following images are seen : (1) On the anterior surface of the cornea—the image is bright and upright ; (2) On the anterior surface of the lens—(near the centre of the pupil)—the image is not so bright but is somewhat larger and upright ; (3) On the posterior surface of the lens—it is small but inverted and not easily seen.

Ask the subject to look at a far wall and then at your finger held near the eye. The image —2 moves closer to image—1, and gets brighter and smaller. This shows that during accommodation the anterior surface of the lens moves forwards i.e., it becomes more convex.

*Experiment No. : 10.6***THE VISUAL ACUITY**

In a patient, the acuteness of vision may be greatly decreased and he may only be able to perceive light and count fingers held in front of his eyes. For more accurate assessment, the distant vision of the subject should be tested with the Snellen's test charts and near vision with Jaeger test types.

A. Distant Vision

The letters on the Snellen's charts are so designed that the letter as a whole subtends an angle of five minutes, and each

part or stroke of the letter substends an angle of one minute on the nodal point. The test chart has a series of printed letters of varying sizes, each line of which, a normal eye should be able to read at definite distances—60, 36, 24, 18, 12, 9 and 6 meters. A subject with normal vision should be able to read the top letter at 60 meters, next line at 36 meters and so on. Ask the subject to stand at a distance of 6 meters (20 feet) from the test type, which should be well illuminated. Then ask him to read the letters from above downwards (the distance for each line, in meter, is mentioned on the chart). If he can read the last line from this distance, his visual acuity is expressed as 6/6. If he can read only the top letter, the visual acuity will be 6/60. Test each eye separately, with and without glasses as the case may be, and express the results as—RE : 6/6 ; LE ; 6/24, etc. In one type of chart, only the letter E or C is printed but pointing in different directions. The subject is asked to indicate the direction in which the letter is pointing. This chart is used when a patient cannot read. Test charts with Punjabi and Hindi letters are also available.

B. Near Vision

For assessing the acuity of near vision, test types of varying sizes, are held at ordinary reading distance. Jaeger's charts are based on printer's 'point' system. Record near vision at the smallest type which a subject can read. Express the results as J_1 , J_2 etc. for each eye separately. J_1 indicates normal near vision.

Visual acuity is a complex phenomenon, and a number of functions are tested when an individual reads the letters on the Snellen's chart. These include the power of attention, the ability to direct the eyes, and to fix them accurately (ocular muscles) so that the image of the letter falls on the most sensitive part of the retina. The accuracy of vision is also influenced by the image forming mechanism of eye, the state of cones and other retinal factors, and the stimulus factors. The last include the illumination i.e., brightness of the letters (stimulus), the contrast between the letters and the background, and the time for which the retina is exposed to the stimulus.

Physiologic Nystagmus : The eyeballs are not absolutely still even when a person stares fixedly at a stationary object. The eyes show continuous, small jerky movements. This physiologic nystagmus, apparently, has the function of continuously and rapidly shifting the retinal images from one receptor to another receptor when continuous visualization of an object is attempted. Saccades (fast movements) involve conjugate movements of the eyes, and are made in reading, and in examining a stationary environment. Smooth pursuit movements, on the other hand, are slow, and are used for tracking a moving object to keep it at the fovea. Frontal eye-fields (area 8) control the saccades.

Experiment No : 10.7

PERIMETRY (FIELD OF VISION)

Principle. The area of the external world visible to a person when he fixes his gaze on an object with one eye is called the field of vision for that eye. The process of charting the field of vision is called perimetry.

Apparatus. Priestley-Smith's and the Lister-type perimeters are self recording. A simple 'hand' perimeter is also available.

The apparatus consists of : (1) A broad metal arc which can be rotated around its centre, clockwise or anticlockwise, so as to describe a hemisphere. It is mounted on a heavy stand in front of a large black disc. A small, round, plane mirror is fitted in the centre of the arc. In some instruments, there is a spring lock at every 15° on the rotation of the arc. (2) Adjustable chin rest mounted on a pillar. (3) Frame for keeping the chart in position. (4) Source of light. This is fitted at the end of one limb of the metal arc, and carries a 100 watt bulb. The light rotates with the arc. (5) Test objects of different colours and diameters, usually 3, 5, 10, 15, 20 millimeters, can be fitted

into a carrier which moves in a groove in one limb of the metal arc which is graduated from 0° — 90° . The test object is moved with a knob, and this also causes the movement of a pin on the back of the apparatus.

In this simple model, the inclination of the arc is read from a plastic dial on the stand behind the plane mirror, and the position of the test object (a white spot painted on the flattened end of a metal rod) is recorded from a scale engraved on the arc itself (Fig. 10.2). The readings are then transferred to correspond-

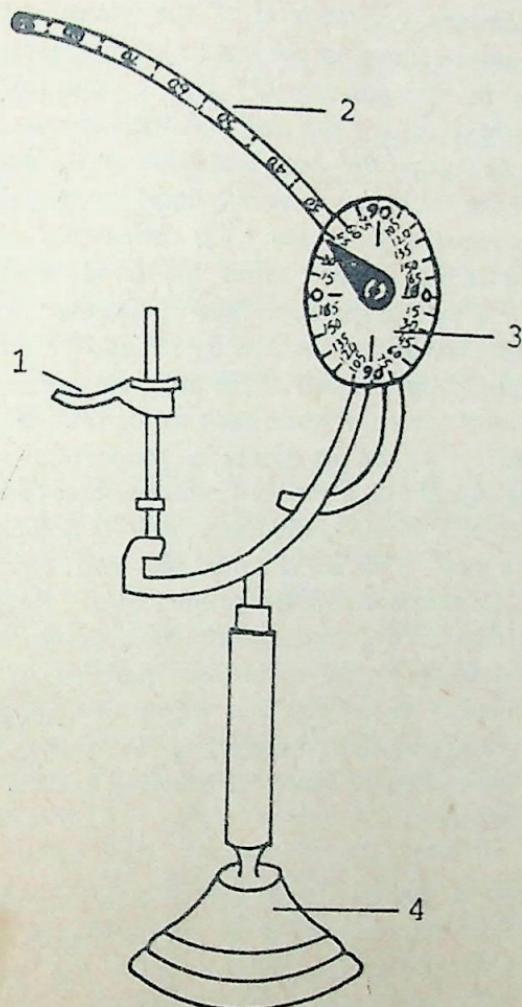


Fig. 10.2 : Student perimeter. 1—adjustable chin rest ; 2—rotatable arc
3—dial ; 4—base.

ing points on the printed perimeter chart. The chart corresponds to the entire field which is divided by circles from 0° to 90° . Meridians, at intervals of 15° are also indicated over the entire field. The limits of the average normal field of vision (peripheral field) for either eye are indicated on the chart for comparison with the charted field of vision.

Procedure : (1) Place the perimeter on a table of suitable height and ask the subject to sit on a stool in front of the apparatus. Fix a chart in the frame provided for this and switch on the light. Adjust the height of the chin rest so that the eye (right eye, for example) is at the level of the plane mirror. Instruct the subject not to move his eye but to keep looking at the image of his eye in the mirror. (2) Fix a 3 mm test object in the carrier and take it to the end of the arc by manipulating the knob. Start on the temporal side in the horizontal meridian. Tell the subject to raise his hand or say 'yes' as soon as the object comes into view. (3) Bring the object inwards and as soon as the subject raises his hand, strike the chart-holder against the pin gently so that it punches a hole in the paper. Move the arc downwards by 15° and gradually bring the object inwards from the end of the arc once again. Repeat the procedure until the arc returns back to the original position (i.e., through 360°). As the pin moves in proportion to the movement of the test object, a series of punched holes are obtained on the chart paper. Remove the chart from its holder, and join the holes with a pen. This is the peripheral field of vision of the right eye. Compare it with the printed field. Record the field of vision of the left eye on the same chart, taking care that the chart is fixed in the holder in correct position. (4) Chart the peripheral field of vision with test objects of blue, green, and red colours. (5) In addition to recording the peripheral field, examine the entire field of vision by bringing the test object right upto the mirror.

Observations and results : It will be seen from the chart that peripheral field extends beyond 90° on the temporal side, about 50° in the vertical meridian, about 55° — 60° on the nasal side, and about 65° downwards. The peripheral field of vision is

maximum for white objects, and smaller for blue, red, and green, in that order. It is essential that the sensation must be more than just an awareness of the presence of some object in the visual field. The subject should be able to distinguish the colour with certainty.

Discussion : Images of objects falling upon the macula are seen in very minut details, and the colours are bright and distinct. Objects visible away from the point of fixation become less and less distinct, and it becomes more difficult to identify the colours.

The visual fields of the two eyes overlap, the portion common to both eyes having a diameter of about 120° . The images falling on the two maculae of the eyes are slightly different from each other. This is the basis of binocular vision which is responsible for depth perception.

The central visual fields are mapped on a black felt screen (Bjerrum's screen). A white target is moved across it and by noting the points where it disappears and reappears, the blind spot (optic disc), and any scotomas (blind spot produced by disease) can be outlined.

Visual pathway. The optic nerve fibres from the nasal half of each retina cross in the optic chiasma, while fibres from the temporal halves remain on the same side (Fig. 10.3). For example, the fibres in the right optic tract are derived from the temporal half of right retina and nasal half of left retina, thus subserving nasal field of vision in right eye, and temporal field of vision in left eye. Optic tract fibres end in two main areas—(a) Some fibres and/or collaterals leave the tract in front of the lateral geniculate body to end in the pretectal region of the midbrain to synapse with the Edinger-Westphal nucleus of the 3rd nerve (some fibres crossing to the opposite nucleus). These fibres are concerned with direct and consensual light reflexes. A lesion in this region produces the Argyll Robertson pupil, in which, the light reflex is lost while the accommodation reflex is present. (b) Other fibres from the optic tract relay in the six layers of lateral geniculate body; fibres from the retina

of the same side end in layers 2,3 and 5, and those from the retina of the opposite side end in layers 1,4, and 6. From the lateral geniculate body, the fibres, after relaying, pass back in the geniculocalcarine tract (optic radiation) through the internal

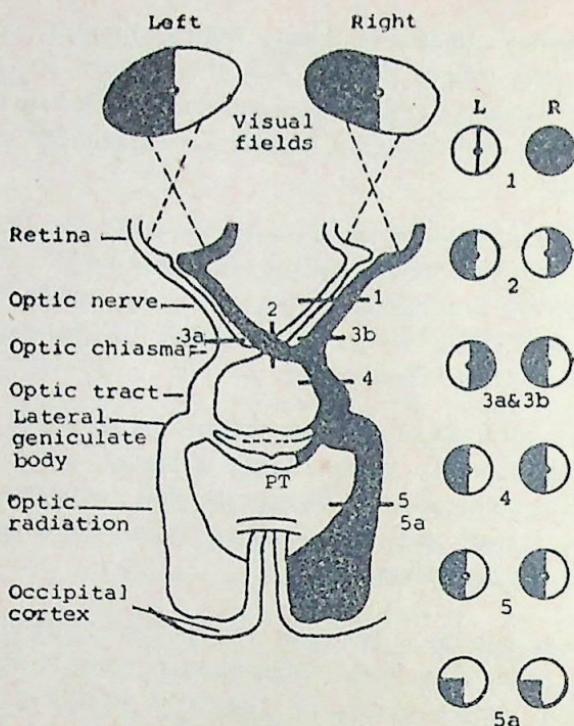


Fig. 10.3 : The visual pathways. Effects of lesions at various locations of the right pathway are shown on the right. L and R—fields of vision, including macular vision. The fibres concerned with light reflex leave the optic tract before the lateral geniculate body, to end in the pretectal region (PT) of the midbrain on the Edinger-Westphal (oculomotor) nuclei. See text for effects of lesions.

capsule (lying behind the fibres for general sensations) and reach the primary visual area i.e., Brodmann's area 17, on the medial aspect of occipital lobe. Areas 18, and 19 on the lateral surface of cerebrum are the association areas.

Lesions of the visual pathway. These produce defects of vision depending on the location and extent of injury (Fig. 10.3).

1. *Lesion of the optic nerve.* Complete blindness in that eye with loss of direct light reflex. Injury to optic nerve, or tumour involving it, produce this type of lesion (lesion at 1 in Fig. 10.3).
2. Circumferential blindness (tubular vision). The causes include hysteria and optic or retrobulbar neuritis.
3. Lesion in the middle of optic chiasma produce bitemporal hemianopia; temporal fields of vision of both eyes affected (as the nasal fibres from both retinae are damaged). Commonly, the lesion is due to a pituitary tumour (lesion at 2).
4. Lesion on one side of optic chiasma, due to calcified internal carotid artery, produces right or left nasal hemianopia. (lesion at 3a or 3b).
5. Lesion on both sides of the optic chiasma by calcified arteries produce binasal hemianopia. (lesion at 3a and 3b).
6. Lesion of optic tract (right, for example), will produce left homonymous hemianopia (same side of both visual fields affected) with hemianopic pupillary response. Tumours of parietal or temporal lobes pressing on the tract are the common causes. (lesion at 4)
7. Lesion of the optic radiation (right, for example) produces left homonymous hemianopia with normal pupillary reaction to light. Complete involvement of optic radiation will produce such visual defects. (lesion at 5).
8. Partial involvement of optic radiations will produce superior (lower portion of radiation involved), or inferior (upper portion of optic radiation involved), homonymous quadrantanopia. (5a).
9. Lesions of the association visual areas on the lateral surface of the brain result from head injuries, and may affect higher visual functions like depth and distance perception, visual localization in space, and objects of daily use may not be recognised.

Precautions. (1) There should be enough light for the subject to visualize the test object.

(2) The eye must not move during the charting of the field of vision.

Questions : (1) What is meant by field of vision ? What is confrontation test ? (2) What is the extent of normal field of vision in the four cardinal directions ? To which side the field is maximum and why ? (3) What is physiological blind spot ? How can its presence be demonstrated ? (4) Trace the visual pathway from the retina to the visual cortex. What will be the effects of lesions at various locations along its path ? (5) Which portion of retina is tested by perimetry ? (6) Is the field of vision the same for all colours ? (7) What is ophthalmoscopy ? What structures can be seen with the ophthalmoscope ? (8) What are muscae volitantes and how are they caused ?

Experiment No : 10.8 **COLOUR VISION**

Colours have three-fold attributes: hue, intensity, and saturation ; the last refers to the degree of dilution of a colour with white. These three independent quantities in colour vision are referred to as trichromy. It is well known that the sensation of any spectral colour can be produced by mixing varying proportion of the three primary colours i.e., *Blue* (wavelength = 450—492 m μ), *Green* (492—575 m μ); and *Red* (647—723 m μ).

Many colour blind subjects, even with severely limited colour vision are, frequently, unaware of their defect. In familiar conditions, they learn to identify objects from their shapes etc., and use other alternative clues. The condition is often unmasked when their colour sense is tested for jobs like engine drivers aviators and sailors.

Testing for Colour Vision

A. Holmgren's wools. Mention has already been made of this test in expt. 9.8. This test may, however, fail to detect colour blindness in some subjects.

B. Ishihara's charts. There is a series of plates, all with splashes of colour in irregular patterns, the colours differing from plate to plate. The figures and letters are intentionally formed of colours which are likely to look the same as the background (See 9.8 also).

C. Edridge-Green's lantern. Differently coloured glass pieces are fitted in metal frames and any colour can be brought in front of the source of light. The size of the aperture through which the coloured glass is seen can also be varied. In addition the lantern has a series of modifying glasses by means of which effects of rain and fog or mist can be imitated.

Experiment No : 10.9**STEREOSCOPIC VISION**

1. Thread a needle first with both the eyes open and then repeat the experiment with one eye closed. Note how long it takes in either case. Your hands should not touch each other while threading the needle, otherwise, the tactile clues will reduce the effect of closing one eye.

2. Hold a matchbox, about 10 to 12 inches in front of your eyes. Draw its appearance as seen with the right eye only and then with only the left eye. Compare the two sketches. The neural mechanisms of vision fuse the two slightly different images into one to give an impression of solidity.

*Experiment No : 10.10***DOMINANCE OF THE EYE**

Just as we habitually use one hand more than the other, we unconsciously favour the use of one eye or the other. Your dominant eye is the one that you normally employ to thread a needle or to look into a camera. You may try the following simple experiment to determine whether you are right-eyed or left-eyed. Make a circle with your thumb and index finger and look through it with both eyes at a small object across the room, say a door handle. Close one eye and then the other ; the eye that sees the object within the circle is your dominant eye.

*Experiment No : 10.11***SUBJECTIVE VISUAL SENSATIONS**

Close one eye and look at the sky, on a clear day, with the other and try to concentrate on what you see. You will observe small circular, grey specks, or zigzag wispy filaments or hair-like objects, or rows of cells, that drift across your field of vision. These are called *muscae volitantes* or *floaters*. If you try to focus your eye on them they drift away or sink down and if you jerk your eye up they move up accordingly only to sink down or drift away once again. They are a normal phenomenon, especially as age advances. They are the shadows cast on the retina by cellular debris in the aqueous and vitreous humors. Images of the retinal vessels reflected from the posterior surface of the lens also contribute to the floaters.

Experiment No : 10.12
OPHTHALMOSCOPY

Principle : An ophthalmoscope is employed to study the structures inside the eye because they are not visible to the naked eye. A beam of light is directed into the subject's eye through the pupil to illuminate the back of the eye (fundus oculi; fundus is that part of the retina that can be seen with an ophthalmoscope) and the observer examines the optic disc, retinal blood vessels, macular region and the peripheral part of the retina. Any opacities in the media can also be visualized.

Apparatus : The self-luminous bifocal ophthalmoscope (Fig. 10.4) contains electric batteries in its handle. The sight hole (viewing aperture) is divided into two parts- a beam of light passes into the subject's eye through the lower part while the observer looks into the eye through the upper half of the aperture. There is a serrated disc the rotation of which can bring a convex or a concave lens of known power in front of the sight hole. The diopteric power of the lens in use is indicated on the back of the instrument. The amount of light being directed into the eye can also be controlled by rotating a ring provided for the purpose.

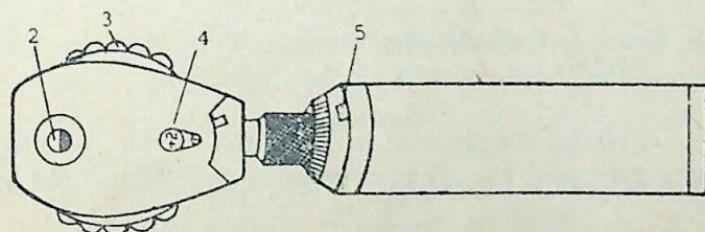


Fig. 10.4 : The ophthalmoscope. 1—handle ; 2—sight hole ; 3—notched disc ; 4—aperture to indicate the power of lens in use ; 5—on-off switch, the amount of light can be controlled by rotating this ring.

Procedure : Routine examination can be carried out without dilating the subject's pupil but for a comprehensive examination of the eyes the pupils should be dilated with 2% homatropine.

pine drops instilled in the conjunctival sac a few minutes before the examination ; care must be taken in cases of glaucoma or when intraocular tension appears to be raised.

(1) Seat the subject on a stool in a dark room. Ask him to look straight ahead at a distant object (this will relax his accommodation) and to keep his eye as still as possible.

(2) Start the examination with the lens in the ophthalmoscope at O position. Switch on the light and while looking through the sight hole approach the subject as close as possible. Relax your own accommodation. Your eye will receive the emergent rays from the subject's eye and if both of you are emmetropic (normal vision ; no error of refraction) you will see the *red reflex*—that is to say the image of the fundus as orange-pink in colour. If either of you is ametropic (some error of refraction) bring the suitable lens in front of the sight hole till the image comes into sharp focus. The image is erect and magnified. If the subject is hypermetropic—the emerging rays will diverge but they can be brought to focus by using a convex lens in front of the sight hole (or you may 'accommodate'). If the subject is myopic, the emerging rays will converge but they can be made more divergent by using a concave lens.

(3) Examine the optic disc, retinal blood vessels, macular region and the peripheral part of the retina.

(4) After the examination is complete, instill a couple of 1% eserine drops in the eye to counteract the effect of the homatropine.

OBERVATIONS, RESULTS, AND DISCUSSION : Though the blood vessels and the optic nerve head (optic disc) can be seen clearly, the retina itself is not visible because it is mostly transparent. The pigment layer of the retina hides the choroid vessels so that the background of the fundus is fairly uniformly orange-pink in colour. This is the so-called 'red reflex' of the fundus. The yellow colour of the macula lutea cannot normally be seen but its location can be inferred from the absence of blood vessels and its location as described below.

(a) *Optic Disc* : This is the region of exit of the optic nerve fibres and entry of blood vessels (the optic nerve fibres leaving the eye become myelinated, and therefore, visible only as they reach the disc). The normal optic disc is round or slightly oval with well-defined edges. It is pale pink and distinctly paler than the surrounding fundus. Its tint, however, varies considerably. It is 1.5 mm in diameter, though of course, it is seen magnified. The central part is somewhat paler and shows a depression—this is the *physiological cup*. The central vessels originate from this lighter area. There are no visual receptors overlying the optic disc and as a result this region is blind as already mentioned in expt. 10.2. In glaucoma, where the intraocular tension is raised, the physiological cup increases in size and retinal vessels kink as they pass over the edge. When the intracranial tension is raised, the optic disc shows swelling (papilloedema) and its outline gets blurred. In optic nerve atrophy, the disc becomes very pale or even greyish white.

(b) *Retinal Blood Vessels* : They are derived from the central artery and vein and radiate out from the optic disc, dividing dichotomously into many branches as they spread out to the periphery.

The arteries are bright red and narrower than the corresponding veins. They show longitudinal silvery stripes due to reflection of light from their convex surfaces. (What we see is the blood column and not the vessel wall which is usually transparent). Veins are purplish and more convoluted. Where the vessels cross, it is normally the arteries that cross the veins. Normally there is no narrowing, change in diameter, direction or colour at the sites of crossings. Normally, the arteries do not show any pulsations; but in aortic regurgitation, there are distinct pulsations visible. The veins may show slight pulsations at or near the edge of the disc in some normal persons. All these points are worth noting since there may be haemorrhages, exudations and other defects in diseases like hypertension, chronic nephritis, diabetes mellitus, and purpura.

The retinal vessels supply the inner layers of retina (bipolar and ganglion cells) whereas the rods and cones (located in the

outer layer of retina) obtain their nourishment from the choroidal capillaries. It is for this reason that retinal tears and detachment lead to blindness.

(c) **Macular Region** : It is located at the posterior pole of the eye, 3 mm (two optic disc diameters) to the temporal side of the edge of the optic disc. It is slightly darker than the surrounding retina. There are no blood vessels. There is a small depression at the centre—the fovea centralis—that is lighter in colour and often glistens. The fovea centralis is the thinned-out rod-free portion of the retina where cones are densely packed and where there are very few cells overlying the receptors. The fovea is the region of most acute vision and is most highly developed in man.

(d) **Periphery of the Fundus** : This area can be visualized only when the pupil is fully dilated. Certain diseases like retinal tears and detachment, and retinal pigmentation start here.

Hearing

INTRODUCTION

Sound is the sensation produced when alternate condensation and rarefaction phases of air (waves or vibrations) strike the tympanic membrane. Hearing is one of our major means of communication with others and has, therefore, considerable psychosociological significance.

The auditory analyser perceives 3 properties of sound, namely *pitch*, which is a psychological perception of sound frequency ; *intensity* or *loudness*, which is a psychological term referring to the amplitude of sound waves ; and *timbre* or *quality*, which refers to the sensation perceived when we hear a mixture of related frequencies (i.e., harmonics or overtones).

The intensity or loudness of sound is expressed in decibels (db). The standard sound reference corresponds to 0 db at a pressure level of 0.0002 dyne/cm². This is the minimum sound intensity that can be perceived by a normal person. Ordinary conversation at 6 to 8 feet is held at 50-60 db (the decibel scale is logarithmic).

The human ear can hear sounds with a frequency range of 16-20,000 Hz (hertz ; 1 Hz = 1 cycle per second). The term *infrasound* refers to frequencies below 20 Hz and *ultrasound* refers to those above 20,000 Hz. The human ear is most sensitive to frequencies between 1000 and 3000 Hz. The pitch of

average male voice in conversation is about 120 Hz and in females it is about 250 Hz. The human ear cannot perceive ultrasounds while bats, dogs and some other animals can (bats use pulses of ultrasound as a form of radar). The echoes from ultrasound are nowadays used as a diagnostic aid in the study of heart valves and other viscera and for detecting foetal abnormalities.

The speed of sound is about 770 miles per hour. The term *supersonic* refers to an object (e.g., an aeroplane) that travels at a speed faster than that of sound. **OSSICULAR, AIR AND BONE CONDUCTION.** In order to understand the basis of 'tuning fork tests' the student should know what is meant by *air conduction* and *bone conduction*. Normally, most of the energy of incident sound waves is conducted to the oval window of the cochlea via the tympanic membrane and middle ear ossicles ; this mode of sound conduction is called *ossicular conduction*. However, it is commonly, though misleadingly, termed air conduction. Sound waves striking the tympanic membrane also set the round window of the cochlea into vibration directly through the air in the middle ear ; this is true *air conduction* although it does not play role in normal hearing. Since the cochlea is enclosed in a bony cavity in the temporal bone, vibrations of skull can also be transmitted to the organ of Corti—the receptor for hearing. This type of sound conduction is called *bone conduction*. But even loud sounds do not possess enough energy to contribute significantly to the process of hearing. Sound from a vibrating tuning fork applied directly to the skull can, however, be heard.

DEAFNESS Deficient hearing may result from a defect in the conduction of sound up to the sensory hair cells of the cochlea (conduction deafness) or from a lesion of the auditory pathway from hair cells to the cerebral cortical area for hearing. *Conduction deafness* may result from wax or a foreign body in the external meatus, thickening or perforation of tympanic membrane or damage to the ossicles by infection, or from fixation of stapes in the oval window. *Nerve deafness* may result from hereditary or toxic degeneration of hair cells due to measles or prolonged use of streptomycin, gentamycin or quinine. Continued exposure to high levels of sounds (90 db and above) can also cause permanent damage to hair cells of cochlea.

Experiment No : 11.1
TESTS OF HEARING

Test each ear separately. Confirm that the external auditory meatus is wax-free before proceeding with the tests.

1. **Whisper test.** Stand on one side of the subject and close his opposite ear with your finger. Ask his name, age, the nature of his work and about his illness, by gently whispering into his ear from a distance of 10-12 inches. Repeat the procedure on the other ear. This test will provide an approximate idea about the faculty of hearing.

2. Stand behind the subject and ask him to close his eyes. Close his left ear with your finger and bring a 'ticking' watch gradually towards his right ear, beginning beyond the probable range of hearing. Ask him to raise his finger when he can just hear the ticking. Repeat the procedure on the left ear. One needs to know, obviously, the distance at which ticking should be audible to the normal ear. You may compare the subject's hearing with your own (presuming your own hearing power to be normal).

(3) **Tuning fork tests.** The student should perform the Rinne and Weber tests on himself and on his partner.

(a) *Rinne's test* : Set a tuning fork into vibration by striking a prong on the heel of your hand and place its base on the mastoid process (the bony prominence behind the ear). You will hear a sound. After a few seconds the sound will stop. Now bring and hold the fork near the ear—the sound will reappear. This shows that in a normal person air conduction of sound is better than bone conduction. This is the basis of Rinne test.

(b) *Weber's test* : Place the base of a vibrating tuning fork on your forehead or on the top of the head. You will hear sound equally well in the two ears. Now close one ear with a finger—the sound will appear louder in that ear. Closing the ear

creates a situation of conduction type of deafness and demonstrates the masking effect of sounds in the environment. Repeat the test on the other ear.

(c) *Schwabach's test* : In this test the bone conduction of the patient is compared with that of a normal subject. Perform this test on your partner after he closes one ear with a finger.

In conduction deafness air conduction is impaired while in nerve deafness both air conduction and bone conduction are defective. The results of tuning fork tests are summarised in table 11.1.

TABLE 11.1 Tuning fork tests to distinguish between conduction and nerve deafness.

	Rinne	Weber	Schwabach
Normal	Vibrations in the air are heard after bone conduction is over.	Sound heard equally well on both sides.	
Conduction deafness (one ear)		Vibration in air are not heard after bone conduction is over.	Sound is louder in the diseased ear (masking effect of noise is absent)
Nerve deafness (one ear)		Vibrations in air are heard after bone conduction is over.	Sound is louder in the normal ear.

*Experiment No : 11.2***LOCALIZATION OF SOUND**

Ask the subject to close his eyes. Use a forceps to produce clicking noises behind the subject and ask him to locate the direction of the sound.

The ability to judge the position of the source of a sound with both ears is called binaural effect. Two factors are involved in this process :

1. Difference in the loudness of sound at the two ears, and
2. The difference in the interval of sound at the two ears i.e., the phase difference or the interval between equal phases of sound waves entering the two ears.

When we attempt to locate direction of sound, we turn the head from side to side until the sound seems to be equally loud in both the ears; the sound then lies directly ahead.

The judgement of the direction of a sound, and the distance of its source, is a complex process where the cortices of the two hemispheres play an important rôle.

*Experiment No : 11.3***MASKING**

That we raise our voice while travelling in a noisy bus is a common experience. When the noise stops suddenly, we become aware of the loudness of the voice. This masking effect of noise is employed to detect malingering.

Ask the subject to read from a book. After a few seconds, make a rattling noise near his ear by using a tin box containing some metal objects. The subject increases the intensity of his voice. Obviously, this would not occur in a deaf person. A person malingering deafness, on the other hand, will raise his voice.

Questions : (1) What is the difference between bone conduction and air conduction ? (2) Place the stem of a vibrating tuning fork on your mastoid process. When the sound can no longer be heard bring it near your ear. What is the result? Explain your findings. (3) Place the stem of a tuning fork on your forehead. Is the sound equally heard on both sides ? Close one ear with a finger and note the effect. Explain your findings. (4) What is organ of Corti ? Describe its structure and mode of functioning. (5) Describe the auditory pathway from the cochlea to the cortical area of hearing. (6) What is the difference between the terms supersonic and ultrasonic ? What is the upper limit of hearing frequency in man ? Is it different in some animals?

APPENDIX

UNITS AND MEASURES EMPLOYED IN PHYSIOLOGY

The international system of units (SI Units—Systems International d'Unites).

Examples of Basic SI Units

<i>Physical quantity</i>	<i>Name of SI unit</i>	<i>Symbol of SI unit</i>
length	metre	m
mass	kilogram	kg
amount of substance	mole	mol
energy	joule	J
pressure	pascal	Pa
time	second	s

Decimal multiples and submultiples of the units are formed by the use of prefixes :

<i>Multiple</i>	<i>Prefix</i>	<i>Symbol</i>	<i>Submultiple</i>	<i>Prefix</i>	<i>Symbol</i>
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^9	giga	G	10^{-6}	micro	μ
10^6	mega	M	10^{-9}	nano	n
10^3	kilo	k	10^{-12}	pico	p
10^2	hecto	h	10^{-15}	femto	f
10^1	deca	da	10^{-18}	atto	a

Units of Volume : The SI unit is the cubic metre (1,000 litres). Because of its inconvenience the litre (l) is used as the unit of volume for most applications in physiology and biochemistry.

$$1 \text{ dl} = 100 \text{ ml}$$

$$1 \text{ m}^3 = 1000 \text{ l}$$

$$1 \text{ fluid ounce (oz)} = 28.41 \text{ ml}$$

$$1 \text{ pint} = 20 \text{ fluid ounces} = 568 \text{ ml}$$

$$1 \text{ gallon} = 4.55 \text{ l}$$

$$1 \text{ cubic inch} = 16.39 \text{ ml}$$

$$1 \text{ cubic foot} = 28.32 \text{ l}$$

Unit of amount of substance : ('Molar') (e.g., mol/l; $\mu\text{m}/\text{l}$) is used for substances of defined chemical composition. It replaces equivalent concentration (mEq/l) which is not part of the SI system for measurement of sodium, potassium, chloride and bicarbonate (the numerical value of these four measurements is unchanged because the ions are univalent).

Mass concentration (e.g., g/l; $\mu\text{g}/\text{l}$) is used for all protein measurements for substances which do not have a sufficient well defined composition and for plasma vitamin B¹² and folate measurement. The numerical value in SI units will change by a factor of 10 in those instances previously expressed in terms of 100 ml (dl). Haemoglobin is an exception. It is agreed internationally that meantime haemoglobin should continue to be expressed in terms of g/dl (g/100 ml). Non SI units are employed for enzymes and immunoglobulins. The katal is the suggested unit to define the catalytic amount of an enzyme. It is defined as the catalytic amount of any catalyst (including an enzyme) that catalyses a reaction rate of mole per second in and assay system.

Unit of Length : The SI unit is the metre (m)

1 Angstrom unit (A) = $10^{-10} \text{ m} = 0.1 \text{ nm}$ (obsolete)

1 micron (μ) = $10^{-6} \text{ m} = 1 \mu\text{m}$ (μ is obsolete and in its place μm is employed)

1 millimicron ($m\mu$) = 10^{-9} m = 1 nm

1 inch (in) = 2.54 cm = 0.0254 m

1 foot (ft) = 0.3048 m

1 yard (yd) = 0.9144 m

1 mile = 1760 yd = 1.609 km

1 nautical mile = 1.852 km

Units of Mass : The SI unit is the kilogram (kg)

1 kg = 1000 grams (g)

1 ounce (oz) = 28.35 g

1 pound (lb) = 16 oz = 453.6 g

1 ton = 2244 lb = 1016 kg

1 tonne = 1000 kg = 0.984 ton = 2204 lb

Unit of Area : The SI unit is the square metre(m^2)

1 square inch = 645.2 mm²

1 square foot = 0.093 m²

1 square yard = 0.836 m²

1 acre = 4840 sq yd = 4047 m² = 0.4047 hectares

1 hectare = 10^4 m² = 2.471 acres

1 square mile = 640 acres = 259 hectares

Unit of Pressure : The SI unit of pressure is the pascal (Pa). This is the pressure exerted by 1 newton on an area of a square metre (1 Pa = 1 N m⁻²)

1 cm water = 98.1 Pa

1 mm Hg = 1 torr = 133.3 Pa = 0.1333 kPa

1 kPa = 7.50 mm Hg = 10.1 cm H₂O

1 lb/in² = 6.894 kPa

1 millibar (mb) = 0.1 kPa

1 normal atmosphere = 1 bar = 760 mm Hg = 101.3 kPa

1 dyne/cm = 10^{-4} kPa

Temperature The SI temperature scale is the kelvin scale (K) but it is inconvenient to use in medicine the Celsius (formerly centigrade) scale (°C) has been retained.

Degree Celsius = K - 273.15

Conversion from Fahrenheit scale to Celsius scale, use either of the following :

$\frac{9}{5} = F - 32$	$\frac{9}{5}(C + 40) = F + 40$
$^{\circ}\text{C}$ —40 10 20 30 35 37 40 45 100	
$^{\circ}\text{F}$ —40 14 32 50 68 86 95 98.6 104 113 212	

BLOOD

Arterial Oxygen saturation (at rest)—Sea level : 97 per cent ; 5000 ft. 90 per cent ; 15,000 ft. 75 per cent (Adult blood contains 0.3 ml O₂ in physical solution and about 19 ml per cent in chemical combination with haemoglobin).

Carbon dioxide combining power—55.75 vol per cent.

Bleeding time (Duke-finger, ear lobe)—2-6 minutes. Ivy : less than 6 minutes.

Blood volume (Av.)—men : 75 ml/kg ; women : 67 ml/kg. 7.5-8 per cent of body weight.

Clot retraction—Begins in 1 to 3 hours ; complete in 24 hours.

Coagulation time—Capillary blood : 2-5 minutes ; Venous blood (Lee-White) : 5-15 minutes.

Colour and volume index—0.90-1.10.

Erythrocyte sedimentation rate—mm 1st hour : Westergren : M : 3-5 ; F : 7-12.

Wintrobe : M : 2-8 ; F : 2-10.

Fragility of red cells—Haemolysis begins at 0.45-0.38 per cent NaCl ; complete at 0.36-0.3 per cent NaCl.

Haematocrit (PCV)—Men : 40-52 percent ; Women : 37-47 per cent (SI : M : 0.40-0.52 : F : 0.37-0.47).

Mean corpuscular volume—75 to 94 cubic microns (SI : 75-94 fl)

Mean corpuscular haemoglobin—27-32 μg . (SI : 27-32 pg).

Mean corpuscular haemoglobin conc.—32-38 per cent (SI : 32-38 g/dl).

Mean corpuscular diameter—6.9-8.0 microns.

Osmolality (S)—275-295 mOsm/kg water.

pH (reaction) (B, arterial)—7.35-7.45 (SI : H⁺ 44.7-45.5 nmol/l).

Prothrombin time—venous plasma : 15 to 20 seconds (75-125 per cent.)

Plasma volume (Av.)—Men : 44 ml/kg ; Women 43 ml/kg.

Specific gravity—Blood 4.7 ; Plasma 1.8 ; Serum 1.5.

Blood Elements

Erythrocytes—M : 4.75-5.5 millions/c mm (μ l) (SI : $4.75 + 10^{12}/1 - 5.5 \times 10^{12}/1$).

F : 4.8-5.1 millions/c mm (μ l) (SI : $4.8 \times 10^{12}/1 - 5.1 \times 10^{12}/1$)

Life span : 120 days ; foetal rbc : 15-25 days. 1 per cent rbc destroyed per day, equal to daily turnover rate of rbc in 50 ml blood.

Production rate : 42,500/c mm per day ; 3,065 millions/kg/day Surface area : 129-145 sq microns.

Reticulocytes—0.8-1.4 per cent.

Haemoglobin—men : 14-18 g/dl (SI : 2.09-2.79 mmol/l) ; women: 12-16 g/dl (SI : 1.86-2.48 mmol/l) (serum haemoglobin : 2-3 mg/dl) Production rate : 0.12 g/dl blood. Iron content : 0.335 per cent 1 g Hb carries 1.34 ml O₂. At 15.6 g Hb the oxygen capacity is 20.9 vol per cent. Iron turnover : 0.21-0.26 mg/kg/day. Foetal Hb : Maternal blood-1.45 ± 0.54 per cent of total Hb. Cord blood (term preg) : 65.8 ± 1.25 per cent.

Leucocytes—4,000-11,000/c mm (μ l).

Differential leucocyte count—Myelocytes : 0 per cent ; Juv. neutros : 0 per cent ; Band neutros : 0.5 per cent.

	Range	Mean cells/c mm
Segm. neutrophils	40-60 per cent	2000-7000
Eosinophils	1-4 per cent	10-400
Basophils	0-1 per cent	0-100
Monocytes	6-8 per cent	500-800
Lymphocytes	20-40 per cent	1500-3000

Lymphocytes—Life span : 100 to 300 days

Platelets—250,000-500,000/c mm (SI : $250-500 \times 10^9/1$). Life span : 8 to 9 days.

BLOOD CONSTITUENTS BIOCHEMICAL VALUES

B—Blood P—Plasma S—Serum U—Urine F—Faeces dl= decilitre = 100 ml=per cent

Determination	SI Units 1	SI Units 2	Other Units 3	Multiplication factors for converting from other units to SI units 4
Acetone and acetoacetate (S)	3-20 mg/1		0.3-2 mg/dl	
Aldolase (S)			3-8 units/ml (Sibley-Lehninger)	
			(Warburg and Christian) Men : 33 units ; Women : 19 units	
Amino acid				
nitrogen (P)	2.1-3.9 mmol/l		3-5.5 mg/dl	
Ammonia (B)	22.16-38.78 μ mol/l		40-70 μ g/dl	0.587
Amylase (S)	2.48-5.58 μ kat/l		80-180 Somogyi units/dl (0.8-3.2 IU/l)	
α_1 -Antitrypsin (S)			210-500 mg/dl	
Ascorbic acid (P)	23-85 μ mol/l		04-1.5 mg/dl	56.8
Base, total serum (S)	145-160 mmol/l		145-160 mEq/l	No change
Bicarbonate (S or P)	24-32 mmol/l		24-32 mEq/l	No change
Bilirubin, total (S or P)	5-17 μ mol/l		0.3-1/mg/dl	17.1
direct (S or P)	1.7-6.8 μ mol/l		0.1-0.4 mg/dl	

(1)	(2)	(3)	(4)
Caeruloplasmin (S)	300-600 mg/l	30-60 mg/dl	10
Calcium, total (S)	2.12-2.62 mmol/l (varies with protein concentration)	8.5-10.5 mg/dl	0.250
Calcium, ionized (S)	1.05-1.3 mmol/l	4.25-5.25 mg/dl	
Carbon dioxide, P_{CO_2} (S or P)	5-6.1 kPa	34-46 mm Hg	0.133
Carotenoids (S or P)	0.9-5.6 μ mol/l	50.300 μ g/dl	0.0186
Vitamin A (S)	0.84-2.10 μ mol/l	24-60 IU/dl (μ g/dl)	
Catecholami- nes (U) (as adrenaline)	0.05-0.55 μ mol/24h	10-100 μ g/24h	0.00546
Chloride (S)	95-105 mmol/l	350-375 mg/dl (95-105 mEq/l)	
Cholesterol (S)	3.6-7.8 mmol/l	140-300 mg/dl	0.0259
Cholesterol esters (S)	65 to 75% of total cholesterol		
Complement (S)	C3 (B_1C), 100-190 mg/dl ; C4(B_1E), 20-60 mg/dl		
Copper (S or P)	16-31 μ mol/l	100-200 μ g/dl	0.157
Coproporphyrin (U)	0.15-0.31 μ mol/24h	100-200 μ g/24h	0.00153
Cortisol, free (P)	276-690 nmol/l	10-25 μ g/dl	27.6
Creatine (U)	0-400 μ mol/24 h	0-50 mg/24 h	7.66
Creatinine (P or B)	62-124 μ mol/l	0.7-1.4 mg/dl	88.4
Fat, as stearic acid (F)	11-18 mmol/24 h	3-5 g/24 h	3.25

(1)	(2)	(3)	(4)
Ferritin (S)		M : 30-300 ng/ml ; F : 20-120 ng/ml	
Fibrinogen (P)	1.5-4 g/l	150-400 mg/dl	0.01
Folate (S)	3-20 μ g/l	3-20 ng/ml	No change
Glucose (B) (FOLIN) (true)	4.4-6.6 mmol/l 3.3-5.5 mmol/l	80-120 mg/dl 60/100 mg/dl	0.0555

Glucose tolerance : For the proper evaluation of the test, the subject should be normally active and free from acute illness. After ingestion of 75 g of glucose in 300 ml water, normal glucose tolerance is present when the two-hour plasma glucose is less than 140 mg/dl, with no value between zero time and 2 hours exceeding 200 mg/dl.

Haptoglobins (S)

(Hb binding)	0.3-2 g/l	30-200 mg/dl	0.01
Iodine (BEI)	0.24-0.51 μ mol/l	3-6.5 μ g/dl	

Iodine, Protein bound (S) (PBI)

Iron (S)	14-29 μ mol/l	80-160 μ g/dl	0.179
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Note : 500 ml transfused blood supplies about 250 mg iron.

Total iron bind- ing capacity (as iron)

45-72 μ mol/l	250-400 μ g/ dl	0.179
	(percent saturation 20-50 %)	

Lactate (S)	0.4-14 mmol/l	3.6-13 mg/dl	0.111
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Lactic dehydro- genase (LDH) (S)

1.50-3.34 μ kat/l	90-200 IU/l
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Lead (B)	0.5-1.9 μ mol/l	10-40 μ g/dl	0.0483
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(1)	(2)	(3)	(4)
Lipase (S)	0.93-6.96% μ kat/l	0.2-1.5 units (ml of 0.1 N NaOH)	
Lipids, total (S)	4.0-10 g/l	400-1000 mg/dl	0.01
Lipid fractions (S or P)	Desirable levels : HDL cholesterol : >40 mg/dl ; LDL cholesterol : < 180 mg/dl : VLDL cholesterol : < 40 mg/dl (To convert to mmol/l multiply by 0.026).		
Magnesium (S or P)	0.7-1.0 mmol/l	1.8-2.4 mg/dl	0.411
Nitrogen (F)	71-143 mmol/24 h	1-2 g/24 h	71.4
Non-protein Nitrogen (NPN) (S)	14-21 mmol/l	20-30 mg/dl	071.4
Oestriol (U)	7-173 mmol/24 h	2.50 mg/24h	3.47
Oxygen (B) Capacity	0.16-0.24 of volume	16-24 vol%	(varies with Hb conc.)
Arterial content	0.15-0.23 of volume	15-23 vol%	(varies with Hb conc.)
Arterial % sat	0.94-1.00% of total	94-100% of capacity	
Arterial Po_2 (PaO_2)	10.67-13.33 kPa (Sea level)	80-100mm Hg	0.133
Venous content	0.1-0.16 of total	10-16 vol%	
Venous% saturation	0.6-0.85 of total	60-85% of capacity	
17 Oxogenic steroids (U)	35-69 μ mol/24 h	10-20 mg/24 h	
Phosphatase, acid (P)		1-5 units (King-Armstrong)	

(1)	(2)	(3)	(4)
	4.48-17.94 μ kat/l	0.5-2 units% (Bodansky) 0.5-2 units (Gutman)	
	0.90-8.97 μ kat/l	0.1-1 unit (Shinowara)	
	27.5-174.14 μ kat/l	0.1-0.3 unit (Bessy-Lowry)	
Women :	3.34-158.65 nkatal/l	0.2-9.5 IU/l	
Men :	8.35-183.7 nkatal/l	0.5-11 IU/l	
Phosphatase, alkaline (P)	59-153.4 μ kat/l 17.94-40.37 μ kat-l	5-13 units (King-Armstrong) 2-4.5 unit (Bodansky) 3-10 units (Gutman)	
	19.73-47.14 μ kat/l	2.2-86 units (Shinowara)	
	222.4-639.4 μ kat/l	0.8-2.3 units (Bessy-Lowry)	
Children :	27.8-175.14 μ kat/l	0.1-0.63 unit (Bessy-Lowry)	
Adults :	501-1419 nkatal/l	30-85 IU/l	
Phosphate (as inorganic P)			
(S)	0.8-1.4 mmol/l	2.5-4.5 mg/dl	0.323
Phenylalanine			
(S)		0.2 mg/dl	
Phospholipid (S)	1.45-2 g/l	145-200 mg/dl	
Potassium			
(S or P)	3.5-5.5 mmol/l	14-20 mg/dl (3.5-5.5 mEq/l)	
Pregnanidiol (U)	0-3.1 μ mol/24h	0.1 mg/24 h	3.12
Proteins (S)	62-82 g/l	6.2-8.2 g/dl	10
albumin	36-52 g/l	3.6-5.2 g/dl	10
globulins	24-37 g/l	2.4-3.7 g/dl	10
CSF protein	0.1-0.4 g/l	10-40 mg/dl	0.01
Pyruvate (S)	45-80 μ mol/l	0.4-0.7 mg/dl	113
Serotonin (B)		0.05-0.2 μ g/ml	

(1)	(2)	(3)	(4)
Sodium (S)	136-145 mmol/l	310-340 mg/dl (136-145 mEq/l)	
Sulphate (as sulphur) (P or S)	50-150 μ mol/l	0.5-1.5 mEq/l	
Thyroxine iodine (S)	244-465 nmol/l	3.1-5.9 μ g/dl	78.8
Transaminases (S)			
Glutamic-oxalacetic (SGOT)	40.1-323.8 nkat/l	5-40 units (6-25 IU/l)	
Glutamic-pyruvic (SGPT)	40.1-280.7 nkat/l	5-35 units (3-26 IU/l)	
Transferrin (S)	23-45 μ mol	200-400 mg/dl	
Triglyceride (as triolin) (P)	0.28-1.69 mmol/l	25-150 mg/dl	0.0113
Urate (P)	0.12-0.42 mmol/l	2-7 mg/dl	0.0595
Urea (B)	2.5-6.6 mmol/l	15-40 mg/dl	0.166
Urobilinogen (F)	50-504 μ mol/24 h	30-300 mg/24 h	1.68
Vitamin B ₁₂ (as cyanocobalamin)	160-925 ng/l	160-925 pg/ml	No change
Vitamin D (cholecalciferol) (D ₃)	10-80 ng/dl : 1,25-dihydroxy-cholecalciferol : 21-45 pg/ml		
Volume, blood (Evan's blue dye method) : Men	66.2-97.7 ml/kg ; Women	46.3-8.5 ml/kg	
Xylose (B)	0.33-3.33 mmol/l	5-50 mg/dl	0.0667
Xylose (U)	1.3-2.6 mmol	0.2-4.0 g	6.66
Zinc (S)	7.65-22.95 μ mol/l	50-150 μ g/dl	

CARDIOVASCULAR SYSTEM

Venous pressure—80-100 mm water (3-8 mm Hg) measured at median basilic vein at the level of right atrium. In foot, person standing, 70-80 mm Hg ; 0-30 mm Hg on exercise.

Capillary pressure—Arterial end, 32 mm Hg ; Venous end, 12 mm Hg.

Jugular vein—0 mm Hg ; Superior vena cava : 3 mm Hg ; Right atrium : 2-3 mm Hg.

Right ventricle—25/2 mm Hg ; Pulmonary artery : 24/9 mm Hg ; Pulmonary wedge (mean) : 2-12 mm Hg.

Pulmonary capillary pressure—8-10 mm Hg ; Osmotic pressure : 25 mm Hg. No tissue fluid formed.

Left ventricle—120/2 mm Hg : Brachial artery : 120/70 mm Hg.

Rate of blood flow—Carotid artery : 500 ml per min (350 ml through internal carotid, and 150 ml through external carotid).

Cerebral artery—total, 750 ml per min (50-60 ml per 100g tissue pre min).

Coronary—50-120 ml per min per 100 g left ventricle. Represents total left ventricle flow of about 115 ml per min in a 70 kg man.

Hepatic (mean flow, total)—1.0 litre pre min.

Renal blood flow—1300 ml pre min.

Circulation time—Saccharin (arm to tongue) : 14.2 ± 2.4 sec ; Ether (arm to lung) : 4-8 sec ; Fluorecein (arm to conjunctiva) : 7-15 sec ; Radiosodium (arm to foot) : 15-105 sec.

Cardiac index—(cardiac output in litres per min per sq m body surface area : 3.2.

Cardiac muscle oxygen consumption—9 ml per min per 100 g left ventricle.

Rate—Infants : 130/min ; 70-72/min ; women : 78-82/min.

Stroke vol.—60-80 ml ; Cardiac output (at rest) : 4.5-5/min.

RESPIRATORY SYSTEM

Inspired air—O₂ : 20.96 per cent (160 mm Hg) ; CO₂ : 0.04 per cent (0.15 mm Hg) ; N₂ : 79 per cent (600 mm Hg).

Alveolar air—O₂ : 13.2 per cent (101 mm Hg) ; CO₂ : 5.3 per cent (40 mm Hg) ; N₂ : 75.3 per cent (572 mm Hg) ; water : 6.2 per cent (47 mm Hg).

Expired air—O₂ : 15.5 per cent (116.5 mm Hg) ; CO₂ : 3.6 per cent (27.5 mm Hg) ; N₂ : 74.0 per cent (569 mm Hg) ; water 6.2 per cent (47 mm Hg).

Arterial blood—O₂ : 19.3 per cent (100 mm Hg) ; CO₂ : 48 per cent (40 mm Hg).

Venous blood—O₂ : 14.1 per cent (40 mm Hg) ; CO₂ : 52 per cent (46 mm Hg).

Minute ventilation—6-8 litres ; Walking ventilation : 12-19 litres;
Max. vent. vol : 100-140 l/min.

Vital capacity (forced expired vol.)—M : 3.5-5 l ; F : 3-4.5 l ;
FEV₁ : 80 per cent.

Oxygen diffusing capacity—M : 27-42 ml/min/mm Hg ; F : 28-30 ml/min/mm Hg.

O₂ consumption (at rest)—250 ml/min ; CO₂ output : 200 ml/min. Respiratory quotient : 0.8.

NORMAL CSF VALUES

Physical characters—Clear, colourless. Specific gravity : 1.003-1.008 Volume : 100-150 ml.

Pressure (reclining)—Newborn, 30-80 mm water ; Children, 50-100 mm water ; Adults, 70-200 mm water (avg=125) Sitting: 300-360 mm water. Note : Jugular compression produces prompt rise and jugular release somewhat slower fall in pressure (Queckenstedt's sign).

Chloride (as NaCl)—120-130 mmol/l (120-130 mEq/l).

Cytology—0-5 cells/c mm Only lymphocytes normally present.

Glucose—2.8-47 mmol/l (50-85 mg/dl (Draw serum glucose at same time).

Protein (total)—Lumbar, 12-45 mg/dl ; Cisternal, 12-25 mg/dl ; Ventricular, 5-15/dl.

BRAIN

Nerve cells—Total number in human nervous system : 100 billion.

Growth—Rate of growth reaches max. early in life. By 6 months the brain doubles in size ; by 3 years it triples ; at 6 years the brain is 95 per cent of mature size with remaining growth achieved in about equal yearly increments of 10 g until age 20.

Oxygen consumption—Adults : 3.3 ml per 100 g per min or about 1/5 of basal O₂ requirement of the entire body. Children : 5.2 ml per 100 g per min. As much as 50 per cent of a 5 yr old child's basal body O₂ consumption may be accounted for by the brain.

Weight v/s total body weight (per cent)—2nd foetal month-25 ; at birth-10 ; adult-2.

KIDNEYS

Nephrons—One million in each kidney. Average length :~ 55 mm.

Renal fraction (portion of cardiac output passing through kidneys)—Av. 21 per cent ; Range, 12-30 per cent.

Glomerular capillary pressure—60 mm Hg.

Glomerular filtration rate (GFR)—125 ml/min (170 litres per day), over 99 per cent reabsorbed in tubules. Normal day output of urine is two to three times the normal night output. Sp gr of urine, 1.022-1.032 when no fluids taken for 12 hours ; 1.005-or lower after copious water drinking. Fixed sp gr of 1.010-1.012 suggests renal disease.

Dilution test (water test)—Empty the bladder at 8 am and drink 1500 ml water within 45 min. Void after every half hour

till noon. At least one specimen should have a sp gr of 1.003 or less. Total quantity passed should be over 80 per cent.

p-Aminohippurate (PAH) clearance: (RPF)—M : 560-630 ml/min; F : 490-700 ml/min.

Blood urea clearance—60-95 ml when urine flow rate is over 2 ml/min (C_m) 40-65 ml when urine flow rate is less than 2 ml/min (C_s).

Creatinine clearance, endogenous (GFR)—Approximates inulin clearance.

Inulin clearance (GFR)—M : 110-150 ml/min; F : 105-132 ml/min (corrected to 1.73 m^2 surface area).

Filtration fraction (FF)—M: 17-21 per cent; F: 17-23 per cent (FF=GFR/RPF).

Maximum glucose reabsorptive capacity (T_{mG})— M: 300-450 mg/min; F: 250-350 mg/min.

Maximum PAH excreting capacity (T_{mPAH})—80-90 mg/min.

Osmolality—On normal diet and fluid intake : range: 500-850 mOsm/kg water. Achievable range, normal kidney: dilution 40-80 mOsm/kg water (at least 3-4 times plasma osmolality).

ENDOCRINES

Pituitary. Growth hormone (HGH) (S)—Adults : 1-10ng/ml (by RIA, radio immuno assay).

Thyroid stimulating (TSH) (S)— $<10\text{ }\mu\text{U/ml}$.

Follicle stimulating hormone (FSH) (S)—Prepuberal : 2-12 mlU/ml; adult male : 1-15 mlU/ml; adult female ; 1-30 mlU/ml; castrate or postmenopausal : 30-200 mlU/ml.

Luteinising hormone (LH)—prepuberal : 2-12 mlU/ml; adult female : $<30\text{ mlU/ml}$; castrate or postmenopausal : $>30\text{ mlU/ml}$; adult male : 1-15 mlU/ml.

Corticotropin (ACTH) (P)—8-10 am, up to 100 pg/ml.

Prolactin (S)—0-20 ng/ml.

Somatotropin C (P)—0.4-2 U/ml.

Adrenal. Average plasma concentration and daily secretion (mg)

Cortisol—7-18 $\mu\text{g}/\text{dl}$ (20); Corticosterone : 0.4 $\mu\text{g}/\text{dl}$ (3); Deoxycorticosterone (DOC): 0.007 $\mu\text{g}/\text{dl}$ (0.15); Dehydroepiandrosterone (DHEA): av. 45 $\mu\text{g}/\text{dl}$ (M:15; F:10).

Aldosterone—Supine, normal salt intake: 2-9 ng/dl; increased when upright.

Dopamine—<135 pg/ml; Adrenalin:<80 pg/ml; Noradrenalin : < 400 pg/ml.

Thyroid. Thyroxine, free (FT_4) (S)—0.8-2-4 ng/dl.

Thyroxine, total (TT_4) (S)—4-11 $\mu\text{g}/\text{dl}$.

Thyroxine binding globulin (TBG) (S)—2.4.8 mg/dl.

Triiodothyronine (S)—80-220 ng/dl; Reverse triiodothyronine (S):
Adult: 30-80 ng/dl.

Thyroid uptake I^{131} (percentage of administered dose)—Euthyroid: 15-45; Hypothyroid:<15; Hyperthyroid:>45 Calcitonin : (S)—400 pg/ml.

Parathyroid. Parathyroid hormone levels vary with method and antibody. Correlate with serum calcium.

Islets. Insulin: (S)—0.17-1.04 $\mu\text{g}/\text{l}$ (4-25 $\mu\text{U}/\text{ml}$.)

Stomach. Gastrin (S, special handling)—up to 100 pg/ml; Elevated : 200 pg/ml Pepsin—150-450 units/ml (Mirsky). Pepsinogen I:(S) 25-100 ng/ml.

Kidney. Renin activity (P, special handling)—Supine, normal sodium intake : 1-3 ng/ml/h standing, or while on low sodium diet or diuretics: 3-5 ng/ml/h.

Gonads. Testosterone (S)—Prepuberal: <100 ng/dl; adult male: 300-1000 ng/dl ; adult female : 20-80 ng/dl ; luteal phase : up to 120 ng/dl. Daily production rate of testosterone : M: 4-11.8 mg ; F : 0.9-2.8 mg.

Oestradiol (S, special handling)—M : 12-34 pg/ml ; Female, menstrual cycle : 1-10 days : 24-68 pg/ml : 11-20 days: 50-186 pg/ml ; 21/30 days : 73-149 pg/ml. Daily production rate : M : 20-45 μg ; F : 25-50 μg ; Children : 5-15 μg .

Progesterone (S)—Follicular phase : 20-150 ng/dl; luteal : 300-2400 ng/dl ; pregnancy : >2400 ng/dl; males : <100 ng/dl.

Placenta. Oestriol (E_3) (S)—Male and nonpregnant female : < 0.2 μ g/dl.

Chorionic gonadotropin (S)—Normal male and nonpregnant female: None detected.

REPRODUCTION

Male. Prostate—Acid phosphatase : 100-1000 u/g tissue. Prostatic fluid contains amorphous material as well as granular cells, epithelial cells and leucocytes (up to 5 cells per high power field). Amount formed—0.5-2.0 ml/day.

Semen	Successive ejaculates				48h
	1st	12h	24h	36h	
Volume (ml)	3.1	2.2	2.0	2.0	2.4
Sperms					
% normal	82	82	82	82	82
millions/ml	135	108	92	98	99
total (millions)	349	213	162	184	203
% motile					
fresh specimen	73	59	65	71	64
3 hours	70	57	64	67	60
6-12 hours	53	41	33	47	46

Fructose content—40-400 mg/dl. Fructolysis : 1.1-1.9 mg fructose utilized per 10^9 sperm cells in 1 hour at 37°C.

Hyaluronidase content—Specimens containing 100 million sperms/ml contain 1 unit/ml (equivalent to 2.5 mg/ml. Lactic acid : 100-1,000 mg/dl).

Spermatozoa—339,385,500,000 produced by average male between ages 25 and 55. Full maturity (spermatogenesis) attained between 9 and 18 years.

Spermatogenesis—(The process by which spermatogonial stem cells proliferate and transform into spermatozoa). One cycle of the seminiferous epithelium lasts 16 days, and the whole of spermatogenesis consumes about 64 days.

Sperm survival—48 hours in cervical secretion. Frozen (-70°C): 67% survival when quick thawed.

Temperature—Scrotum : 91.8°F ; Inguinal canal : 95.6° F ; testes : 91.6°F .

Testis—Length : 4-5 cm. Testosterone 10 mg produced in 24 hours.

Urethral resistance—80-100 cm water.

Female. Ovary—weight increases to max of 10 g by age 20. Remains at this level till age 30, after which there is a gradual decline to about 5 g by age 50. Approximately, 400 ova produced ; 400,000 follicles undergo atresia.

Ovulation—14 days before the onset of next expected menstruation. Occurs 21 to 28 days after spontaneous abortion ; 40-42 days after full term delivery. Duration of post-pregnancy sterility in nursing women is about 260 days.

Menarche—14.9 years. 75 per cent of girls experience menarche between 9 and 16 years.

Menstrual blood loss—10 to 55 ml per 4 day period.

Menopause—45 to 50 years. Av. 47 years.

Gestation—Variously reported from 267 to 398 days. It has been suggested that the intrauterine phase of human gestation is cut short because of the large size and continuing growth of the brain and that the average infant must be born approx. 267 days after fertilization of the ovum if he has to be born through the pelvis at all.

Amniotic fluid—weeks of pregnancy	12	15	20
amount (ml)	50	125	400

MISCELLANEOUS

Dissipation of body heat--0.01 to 0.1 watt per sq cm body surface.

Vision—Near point recedes steadily with age, 10 diopters at 1 yr to 1-2 diopters at age 50.

Intraocular pressure—(Schiotz tonometer): 25 mm Hg. Readings below 4 on tonometer with 5.5 g weight and below 6 with 7.5 g weight call for further study.

Hair—Life : Scalp : 2 yrs ; Eyelash : 3 mo ; Eyebrow : 3 wks.

Growth rate : Scalp : 0.35 mm per day : beard : 0.2-0.4 mm per day. Loss : Head : 70 g per year Constituents : cholesterol, 4-12 per cent ; squaline, 2-7 per cent ; calcium, 127 mg per cent ; iron, 1.8 mg per cent.

Skin—pH: 4 to 6 ; Electrical resistance : skin resistance to electricity is probably determined by the chloride content of sweat : resistance : $106-868 \text{ ohms} \times 10^3$.

Tears—total osmotic pressure : 0.903 to 1.014 g NaCl/100 g water.

Bile and pancreatic pressure (resting)—Bile duct : 13-22 cm water ; Pancr duct : 16-30 cm.

Intraperitoneal pressure (mm water)—Supine: 8 ; walking : 18 ; coughing : 62 ; semi-Fowler : 10 ; sitting : 17 ; vomiting : 80; defaecation : 34-41 ; full-Fowler : 16.

Paediatrics

Developmental progress

	Av	Late	Very late
Tonic neck reflex	0-3 mo	3-6 mo	6-8 mo
Head from prone	1-2	2-3	3-4
Reaches for objects	3-4	4-5	5-6
Sits (no support)	6-8	8-10	10-12
Crawls	6-7	7-8	8-9
Prehension	9-9½	9½-10½	10½-11
Single words	9-11	11-12	12-13
Stands alone	11-12	12-13	13-14
Walks alone	12-15	15-18	18-20
Speaks in sentences	21-24	24-30	30-33

Neurological examination (newborn infants). The tests should be performed after first 24 hours.

Moro Reflex—Elicited by any sudden stimulus (loud noise, blow to bed etc.). Response consists of abduction and extension of extremities with extension and fanning of digits, except for the distal phalanges of the index finger and thumb which are flexed. This is followed by flexion and adduction of extremities.

Ocular and visual reflex—Eyes close under strong light.

Gustatory reflex—Infant forces salt-coated finger back with his tongue.

MacCarthy reflex—Percussion of superior orbital region causes blinking of homolateral eyelid.

Doll's eye reflex—Lateral rotation of head causes rotation of eyes in opposite direction.

Grasp reflex—Stimulation of palmar surfaces of hand near thumb and index finger causes forceful grasping of hands.

Tonic neck reflex—Firm turning of head to one side is followed by spontaneous increase in muscle tone and extension of both extremities on the side towards which the face is turned and by flexion in the elbow and knee of opposite limb.

Plantar grasp reflex— stroking plantar surface of foot causes flexion of toes.

Vollmer reflex—Vigorous cry, flexion of extremities, lordosis of spine, and elevation of head when infant is supported by the abdomen and is firmly stroked down the spine.

Crossed extension reflex—Infant in supine position, the sole of the foot is stroked by a pin, the opposite leg flexes and extends, with adduction of the leg and extention of toes with fanning.

Babinski reflex—Tactile excitation of the plantar aspect of foot causes extension of great toe and, at times, fanning of the other toes.

